LISTERIA MONOCYTOGENES ON FRESH-CUT PRODUCE: MECHANISMS OF TRANSFER AND ADAPTATION

by

YAN QI

(Under the Direction of Xiangyu Deng)

ABSTRACT

In recent years, *L. monocytogenes* has been frequently implicated in foodborne outbreaks linked to fresh-cut produce. However, little is known about underlying mechanisms of *L. monocytogenes* transfer and adaptation on fresh-cut produce. We first interviewed three retailers and identified that the common use of gloves represented a potential route for cross contamination with foodborne pathogens. Therefore, we investigated and modeled glove-mediated transfer of *L. monocytogenes* onto fresh-cut cantaloupe. Then, we characterized transcriptomic responses of *L. monocytogenes* on fresh-cut apples, cucumbers, cantaloupes, tomatoes, and celery to probe produce factors and bacterial mechanisms that may underlie *L. monocytogenes* adaptation on fresh-cut produce.

Results showed that glove type, contact time, and contact pressure did not have significant effects on *L. monocytogenes* transfer from cantaloupe rind to flesh or from flesh to flesh. However, glove type appeared to affect *L. monocytogenes* transfer from the stem scar tissue to cantaloupe flesh (P=0.0371). Transfer from rind pieces that had been washed with water was significantly higher compared to transfer from pieces that had not been washed (P=0.0006).

Predictive modeling showed that *L. monocytogenes* transfer occurs on pieces of cantaloupe flesh sequentially touched 85 times by a gloved hand.

With transcriptomic analysis, we identified a core transcriptome including commonly up- and down-regulated genes on fresh-cut produce to explore the factors that may affect the fate of *L. monocytogenes* on fresh-cut produce. The up-regulated genes are involved in amino acid metabolism, fatty acid metabolism, and membrane transportation. The down-regulated genes are involved in carbohydrate metabolism and transportation. Also, *L. monocytogenes* was able to make substantial transcriptomic adjustments in response to properties specific to certain produce, such as different abundance of amino acids, carbohydrates, and antimicrobial substance.

The study on glove-mediated *L. monocytogenes* transfer provides insights to conduct risk assessment on the practice used to prepare fresh-cut cantaloupe. Transcriptomic profiling of *L. monocytogenes* on fresh-cut produce provides molecular assessment of metabolic and stress responses that are likely important for the pathogen's adaptation on fresh-cut produce. This study provides potential transcriptomic markers and molecular targets for future development of detection and control strategies of *L. monocytogenes* on fresh-cut produce.

INDEX WORDS: *L. monocytogenes*, Transfer, Cross contamination, Modeling, Gloves, RNA-seq, Adaptation, Transcriptome

LISTERIA MONOCYTOGENES ON FRESH-CUT PRODUCE: MECHANISMS OF TRANSFER AND ADAPTATION

by

YAN QI

B.S., Shenyang Agricultural University, China, 2013

M.S., Illinois Institute of Technology, USA, 2015

A Dissertation Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirement for the Degree

DOCTOR OF PHILOSOPHY

ATHENS, GEORGIA

2019

LISTERIA MONOCYTOGENES ON FRESH-CUT PRODUCE: MECHANISMS OF TRANSFER AND ADAPTATION

by

YAN QI

Major Professor: Xiangyu Deng
Committee: Mark A. Harrison
Ynes Ortega
Wei Zhang

Electronic Version Approved:

Suzanne Barbour
Dean of the Graduate School
The University of Georgia
August 2019

DEDICATION

I dedicate this dissertation to my family members who have given me the courage and support to pursue the Ph.D. degree.

ACKNOWLEDGEMENTS

First and foremost, I would like to thank my advisor Dr. Xiangyu Deng. It was his patient training and guidance that prompted me to assimilate more knowledge and shape my own critical thinking skills. He was always willing to give me a hand when I encountered the difficulties in my academic studies. Secondly, I would like to thank my committee members Dr. Mark A. Harrison, Dr. Ynes Ortega, and Dr. Wei Zhang. I really appreciate they have taken time to train both of my oral and thinking skills. Thanks for attending my committee meeting and final dissertation defense.

Next, I would like to thank Dr. Larry R. Beuchat, Yingshu He, Shaokang Zhang, Shaoting Li, Anna Townsend, and David Mann. They provided a great help in my research. Thanks Wayne Harvester, he was always willing to help me figure out the computer problems and set up the meetings and presentations.

Last but not the least, I would like to thank all my friends. They provided me a great support and comfort on overcoming the difficulties. They brought me a great happiness and courage during my ph.D. study. I really appreciate it.

TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER 1

INTRODUCTION

In recent years, several listeriosis outbreaks in the United States have been linked with fresh and fresh-cut produce consumption. In 2011, contaminated cantaloupes were involved in one of the most severe foodborne disease outbreaks (CDC, 2012). In this outbreak, a total of 147 people became ill, among which 33 died (CDC, 2012). In 2014, listeriosis linked to caramel apples resulted in 7 deaths and 34 hospitalizations (CDC, 2015).

Fresh produce can be contaminated with foodborne pathogens in processing facilities and retailers (Alegbeleye et al., 2018). Foodborne pathogens are able to attach to the surface of fresh produce (Monier & Lindow, 2005). Once attached, pathogens can aggregate on produce surfaces and occasionally transfer to the edible flesh of fresh produce (Monier & Lindow, 2005). Although sanitizing agents can inactivate pathogens on fresh produce surfaces, they have little effect on microorganisms which have already lodged within specific sites of the produce or been transferred to flesh (Erickson, 2012). For instance, the porous, netted rind and stem scar tissues of whole cantaloupes can provide potential niches for bacterial attachment, infiltration, and biofilm formation. Sanitizers such as chlorine cannot completely eliminate the bacteria that have already attached to the surface (Perkins-Veazie et al., 2012; Ukuku, 2006). The use of knives and slicers that are not properly cleaned and sanitized can result in cross contamination of fresh produce with foodborne pathogens (Ukuku, 2006). Except for knife- and slicer-mediated transfer, bacterial transfer between gloved hands and fresh produce was identified as a potential route for cross contamination during processing. Bacteria transfer between glove contact surface

and produce depends on the type of surface, inoculum and moisture level, contact area, and contact time (Brar & Danyluk, 2013; Pérez-Rodríguez et al., 2008). When the fresh produce is contaminated, extrinsic factors such as storage time and storage temperature are important to determine pathogen survival and growth on fresh produce. Danyluk et al. (2014) used a mathematical model and predicted a 4 log and 1 log increase in numbers of *L. monocytogenes* on fresh-cut cantaloupes during 15 days and 6 days of storage, respectively. Luo et al. (2010) reported that *E. coli* O157:H7 on fresh-cut lettuce can survive at 5°C storage and proliferate at 12 °C storage.

L. monocytogenes growth and survival kinetics on fresh produce are well documented (Danyluk et al., 2014; Salazar et al., 2017). However, little is known about molecular mechanisms of the adaptation of *L. monocytogenes* on fresh-cut produce, which can be investigated by transcriptome profiling. Transcriptomic analysis can be used to explore the impact of environmental conditions, stress factors, and chemical stimuli on bacterial gene expression across entire genomes (Mutz et al., 2013). This analysis has been used for characterizing metabolic states, cellular activities, and physiology of bacteria in multiple food matrices (Deng et al., 2012; Fratamico et al., 2011; Palumbo et al., 2005; Tang et al., 2015).

This dissertation is divided into five chapters. Chapter 1 presents an introduction of this dissertation. Chapter 2 reviews literature on related topics. Chapter 3 investigates factors affecting cross contamination of fresh-cut cantaloupe flesh by *L. monocytogenes* via gloved hands during fresh cut processing and used mathematic models to illustrate transfer. Chapter 4 investigates transcriptomic responses of *L. monocytogenes* adaptation on five produce items commonly sold as fresh-cut products (apple, cantaloupe, cucumber, tomato, and celery). Chapter 5 summarizes this dissertation.

References

Alegbeleye, O. O., Singleton, I., & Sant'Ana, A. S. (2018). Sources and contamination routes of microbial pathogens to fresh produce during field cultivation: a review. *Food Microbiology, 73*, 177-208.

Brar, P. K., & Danyluk, M. D. (2013). *Salmonella* transfer potential during hand harvesting of tomatoes under laboratory conditions. *Journal of Food Protection, 76*(8), 1342-1349.

CDC. (2012). Multistate outbreak of listeriosis linked to whole cantaloupes from Jensen Farms, Colorado (final update). *Available at:* https://www.cdc.gov/listeria/outbreaks/cantaloupes-jensen-farms/index.html. *Accessed on August 14th 2018.*

CDC. (2015). Multistate outbreak of listeriosis linked to commercially produced, prepackaged caramel apples made from Bidart Bros. Apples (final update). *Available at:* https://www.cdc.gov/listeria/outbreaks/caramel-apples-12-14/index.html *Accessed on December 26th 2018.*

Danyluk, M. D., Friedrich, L. M., & Schaffner, D. W. (2014). Modeling the growth of *Listeria monocytogenes* on cut cantaloupe, honeydew and watermelon. *Food Microbiology, 38*, 52-55.

Deng, X., Li, Z., & Zhang, W. (2012). Transcriptome sequencing of *Salmonella enterica* serovar Enteritidis under desiccation and starvation stress in peanut oil. *Food Microbiology, 30*(1), 311-315.

Erickson, M. C. (2012). Internalization of fresh produce by foodborne pathogens. *Annual Review of Food Science and Technology, 3*, 283-310.

Fratamico, P. M., Wang, S., Yan, X., Zhang, W., & Li, Y. (2011). Differential gene expression of *E. coli* O157: H7 in ground beef extract compared to tryptic soy broth. *Journal of Food Science, 76*(1), M79-M87.

Luo, Y., He, Q., & McEvoy, J. L. (2010). Effect of storage temperature and duration on the behavior of *Escherichia coli* O157: H7 on packaged fresh-cut salad containing romaine and iceberg lettuce. *Journal of Food Science, 75*(7), M390-M397.

Monier, J.-M., & Lindow, S. (2005). Aggregates of resident bacteria facilitate survival of immigrant bacteria on leaf surfaces. *Microbial Ecology, 49*(3), 343-352.

Mutz, K.-O., Heilkenbrinker, A., Lönne, M., Walter, J.-G., & Stahl, F. (2013). Transcriptome analysis using next-generation sequencing. *Current Opinion in Biotechnology, 24*(1), 22-30.

Palumbo, J. D., Kaneko, A., Nguyen, K. D., & Gorski, L. (2005). Identification of genes induced in *Listeria monocytogenes* during growth and attachment to cut cabbage, using differential display. *Applied and Environmental Microbiology, 71*(9), 5236-5243.

Pérez-Rodríguez, F., Valero, A., Carrasco, E., García, R. M., & Zurera, G. (2008). Understanding and modelling bacterial transfer to foods: a review. *Trends in Food Science & Technology, 19*(3), 131-144.

Perkins-Veazie, P., Beaulieu, J. C., & Siddiq, M. (2012). Watermelon, cantaloupe and honeydew. *Tropical and Subtropical Fruits: Postharvest Physiology, Processing and Packaging. John Wiley & Sons, Inc*, 549-568.

Salazar, J. K., Sahu, S. N., Hildebrandt, I. M., Zhang, L., Qi, Y., Liggans, G., Datta, A. R., & Tortorello, M. L. (2017). Growth kinetics of *Listeria monocytogenes* in cut produce. *Journal of Food Protection, 80*(8), 1328-1336.

Tang, S., Orsi, R. H., den Bakker, H. C., Wiedmann, M., Boor, K. J., & Bergholz, T. M. (2015). Transcriptomic analysis of *Listeria monocytogenes* adaptation to growth on vacuum-packed cold smoked salmon. *Applied and Environmental Microbiology*, *81*(19), 6812-6824.

Ukuku, D. O. (2006). Effect of sanitizing treatments on removal of bacteria from cantaloupe surface, and re-contamination with *Salmonella*. *Food Microbiology, 23*(3), 289-293.

CHAPTER 2

LITERATURE REVIEW

2.1 Microbial food safety of fresh and fresh-cut produce

2.1.1 Fresh and fresh-cut produce and foodborne outbreaks associated with fresh and fresh-cut produce

Fresh produce has become more popular around the world because consumers demand more natural, convenient, healthy, and fresh food (Bhunia, 2018; Dimitri et al., 2003). Fresh produce plays a vital role in daily diets. It is estimated that low fruit and vegetable intake may result in approximately 1.7 million deaths and 16 million disability-adjusted life years (FAO, 2013). Therefore, the World Health Organization (WHO) and Food and Agriculture Organization (FAO) have set the recommendation that a minimum of 400 g of fruit and vegetables should be taken per day to decrease the risk of malnutrition and chronic diseases such as heart disease, diabetes, and cancer (FAO, 2013).

In recent years, foodborne diseases caused by consumption of fresh and fresh-cut produce contaminated with microbial pathogens have increased (Johnston et al., 2005; Van Boxstael et al., 2013). Table 2.1 shows a list of major outbreaks associated with fresh and fresh-cut produce in the past five years (CDC, 2018a). In 2014, consumption of caramel apples containing *L. monocytogenes* resulted in 35 cases of illness and 7 deaths across 12 states. Whole Granny Smith and Gala apples were involved in this outbreak (CDC, 2015). In 2018, a multistate outbreak of *Salmonella* Adelaide infections was linked to pre-cut melon, leading to 77 cases of infection in 7 states (CDC, 2018d). In 2018, a multistate outbreak of *E. coli* O157: H7 infection linked to

romaine lettuce resulted in 210 illnesses and 5 deaths. The source of contamination was traced to canal water in the Yuma, Arizona growing region (CDC, 2018c).

Table 2.1 Foodborne pathogens linked to consumption of fresh and fresh-cut produce from years 2013-2018 in the U.S.

Year	Microorganisms	Source	Cases	Hospitalizations	Death
2018	*Escherichia coli* O157:H7	Romine lettuce	59	23	0
2018	*Escherichia coli* O157:H7	Romine lettuce	210	96	5
2018	*Salmonella* Montevideo	Raw sprouts	10	0	0
2018	*Salmonella* Adelaide	Pre-cut melon	77	36	0
2018	*Cyclospora cayetanensis*	Fresh salad mix	511	24	0
2018	*Cyclospora cayetanensis*	Fresh produce vegetables trays	250	8	0
2017	*Escherichia coli* O157:H7	Leafy greens	25	9	1
2017	*Salmonella* Urbana	Maradol papayas	7	4	0
2017	*Salmonella* Newport & *Salmonella* Infantis	Maradol papayas	4	2	0
2017	*Salmonella* Anatum	Maradol papayas	20	5	1
2017	*Salmonella*	Maradol papayas	220	68	1
2016	*Listeria monocytogenes*	Frozen vegetables	9	9	3
2016	*Listeria monocytogenes*	Packaged salad	19	19	1
2016	*Escherichia coli* O157:H7	Alfalfa sprouts	11	2	0

2016	*Salmonella* Reading & *Salmonella* Abony	Alfafa sprouts	36	7	0
2016	*Salmonella* Muenchen & *Salmonella* Kentucky	Alfafa sprouts	26	8	0
2016	Hepatitis A	Frozen strawberries	143	56	0
2015	*Salmonella* Poona	Cucumber	907	204	6
2014	*Listeria monocytogenes*	Caramel apples	35	34	7
2014	*Listeria monocytogenes*	Bean sprouts	5	5	2
2014	*Escherichia coli* O121	Raw clover spouts	19	8	0
2014	*Salmonella* Newport	Cucumbers	275	141	1
2014	*Salmonella* Enteritidis	Bean sprouts	115	29	0
2014	*Cyclospora cayetanensis*	Cilantro	204	7	0
2013	*Salmonella* Saintpaul	Cucumber	84	17	0
2013	*Cyclospora cayetanensis*	Fresh produce	631	49	0

(Table modified from CDC, 2018)

2.1.2 Fresh produce supply chain

Fresh produce is grown, imported, and exported in large volumes with complex transportation and supply chain units. The traditional fresh produce supply chain typically includes five major steps: 1) fresh produce production in field, 2) postharvest storage, 3) processing, 4) distribution and retail, and 5) consumer handling (Jacxsens et al., 2010).

2.1.2.1 Sources of pathogen contamination in fresh produce supply chain

One of the major sources for pathogen contamination in the field is agricultural water such as irrigation water. Irrigation water can harbor a wide variety of microorganisms that could cause foodborne illness. These microorganisms can survive on the surface of fresh produce (Materon et al., 2007). In addition, the way fresh produce is harvested may cause bacterial contamination. For example, workers in the field wear gloves while harvesting produce and the use of gloves may serve as a potential route for cross contamination (Erickson et al., 2018). Vectors, such as worms and insects, can also transfer foodborne pathogens from soil to produce surfaces (Caldwell et al., 2003). Also, raw manure as fertilizer can shelter foodborne pathogens. These pathogens can survive in soil and contaminate fresh produce (FDA, 2018b).

Foodborne pathogens can also contaminate fresh produce at the post-harvest stage. Normally, harvested produce will undergo a brief wash step by soaking or spraying with sanitizers. The fresh produce will then be sorted, packaged, and transported for retail (Parnell et al., 2005; Soares et al., 2012). Since there are no complete lethality steps applied on fresh produce, there may be chances for bacterial cross contamination during the post-harvest stage (Parnell et al., 2005; Soares et al., 2012). Equipment, such as cutting boards, slicers, knives, and gloves, is commonly used in fresh produce processing. The use of cutting boards is a potential route for cross contamination. For example, *E. coli* O157: H7 can be transferred from contaminated beef to a cutting board. Then, if lettuce is cut on the same cutting board, *E. coli* O157: H7 could transfer from cutting board to lettuce (Wachtel et al., 2003). Foodborne pathogens can also spread on large quantities of fresh produce such as onions, lettuce, and tomatoes during mechanical slicing and cutting (Scollon et al., 2016; Shieh et al., 2014; Zilelidou et al., 2015). Numerous studies have investigated bacterial transfer on meat or fresh produce via gloves (Jimenez et al., 2007; Rebecca Montville et al., 2001; Wu & Ponder, 2018). Jimenez et al.

(2007) reported that transfer events can occur between a gloved hand and a green bell pepper. Wu et al. (2018) investigated *Salmonella* transfer rates from single-use gloves of different materials to dehydrated pork jerky and concluded that transfer rate of bacteria via nitrile gloves was significantly lower than that via polyethylene gloves ($P < 0.05$). They suggested that polarity of the nitrile rubber may interfere with surface charge of *Salmonella* cells, and then keep more cells on gloves surface during bacterial transfer.

2.1.2.2 Pathogen growth and survival in fresh produce supply chain

Storage time and temperature affect the fate of foodborne pathogens on fresh produce at the post-harvest stage. Salazar et al. (2017) investigated *L. monocytogenes* growth on processed produce, such as fresh-cut melons, cucumbers, and olives. The time needed to reach a 1 log increase in *L. monocytogenes* population ranged from ~14 h on red bell pepper to ~102 h on cucumber at 5 °C (Salazar et al., 2017). They also reported that the time to obtain a 1 log increase in *L. monocytogenes* population ranged from ~ 5 h on avocado pulp to ~ 37 h on cucumber at 25°C. Also, Luo et al. (2010) showed that *E. coli* O157:H7 can survive on fresh-cut lettuce at 5°C and proliferate at 12°C. These results indicated that appropriate storage time and temperature are important to ensure food safety of fresh produce.

2.1.2.3 Methods for the control of foodborne pathogens in fresh produce supply chain

The Food Safety Modernization Act (FSMA) final rule on produce safety has set the standard for the safety of fresh produce in fields. For example, the average amount of *E. coli* in water sources should be no more than 126 CFU per 100 ml water (FDA, 2018a). The statistical threshold value (STV) that reflects the amount of variability in the water quality should not exceed 410 CFU per 100 ml water (FDA, 2018a). The produce safety rule has also regulated that

equipment and tools should be stored, maintained, and cleaned properly. Farmers and workers should be trained and experienced in good hygiene (FDA, 2018a).

The U.S. Food and Drug Administration's (FDA) has also released guidance documents that help direct the food safety of fresh produce. For example, produce processors should record and maintain traceback procedures to address food safety problems (FDA, 2008). Workers should follow Good Agricultural Practices (GAPs) and Good Manufacturing Practices (GMPs) to handle fresh produce. Additionally, a cooperation should be established to allow identification of fresh-cut produce from growers to processing operations, to retailers and to consumers (FDA, 2008). Additionally, FDA Food Code (FDA, 2017) has also suggested that certain produce are considered as time and temperature control for safety (TCS) food after being cut. Examples of TCS foods include cut melons and cut leafy greens. TCS foods should be refrigerated at 4°C or lower to prevent potential foodborne pathogens from multiplying or forming toxins (FDA, 2017). Table 2.2 shows the TCS food based on food intrinsic factors (pH and Aw).

Table 2.2 Interaction of pH and Aw for control of vegetative cells and spores in food not heat-treated or heat treated but not packaged

Aw values	pH:< 4.2	pH: 4.2-4.6	pH:> 4.6-5.0	pH:> 5.0
<0.88	Non-TCS Food	Non-TCS Food	Non-TCS Food	Non-TCS Food
0.88-0.90	Non-TCS Food	Non-TCS Food	Non-TCS Food	TCS Food
>0.90-0.92	Non-TCS Food	Non-TCS Food	TCS Food	TCS Food
>0.92	Non-TCS Food	TCS Food	TCS Food	TCS Food

(Table modified from FDA, 2017)

To decrease the likelihood of cross contamination with foodborne pathogens during the post-harvest stage, sanitizing approaches are used. Dish soap or hypochlorite applied on knives can decrease cross contamination between knife and tomatoes (Soares et al., 2012). T-128, a fresh produce washing aid can inactivate *Salmonella* and *Pseudomonas* biofilms on stainless steel in a chlorinated wash solution (Shen et al., 2012). Gaseous chlorine dioxide applied on apples, tomatoes and onions led to substantial reduction in populations of *Salmonella*, *L. monocytogenes*, and *E. coli* O157:H7 (Sy et al., 2005). Levulinic acid (LV) and sodium docecyl sulfate can inactivate *Salmonella* and *E. coli* O157:H7 on gloves. This study also showed that pathogens residing on gloves can be inactivated, but the inactivation varies with glove types (Erickson et al., 2018).

2.1.2.4 Methods for the modeling of foodborne pathogen activities in the fresh produce supply chain

Predictive models can help to address problems such as bacterial survival and growth during food storage and bacterial transfer during mechanical slicing. Predictive models may not be perfect because of their intrinsic inaccuracies, extrapolations, and unexpected biological behaviors (Zwietering & den Besten, 2011). However, predictive models are still widely used (Baranyi & Roberts, 1994; Danyluk et al., 2014; Shieh et al., 2014; Scollon et al., 2016; Salazar et al., 2017). Through predictive modeling, users can draw conclusions and develop strategies to validate the food safety process (Guillier, 2016).

One type of model used for predicting bacterial growth on fresh produce is the Baranyi model (Baranyi & Roberts, 1994; Vandamm et al., 2013; Salazar et al., 2017). Vandamm et al. (2013) used the Baranyi and Roberts model to study growth rates of *L. monocytogenes* on fresh-cut celery. They found that growth rates of *L. monocytogenes* in cut celery ranged from 0.07 to

0.15 log CFU/g/day at 12°C (Vandamm et al., 2013). Also, Salazar et al. (2017) used this model to determine the growth kinetics of *L. monocytogenes* in cut produce. Based on this model, they reported that *L. monocytogenes* showed high rates of growth on avocado pulp and cantaloupe flesh (Salazar et al., 2017).

Models used for predicting transfer of bacteria on fresh produce are the exponential decay model, the logarithmic model, and the power model (Scollon et al., 2016; Shieh et al., 2014). For example, Scollon et al. (2016) used the exponential decay model to illustrate the transfer of *L. monocytogenes* during onion slicing. They found that this model showed good performance for describing the bacterial transfer behaviors on onions (Scollon et al., 2016). Shieh et al. (2014) used the power model and the logarithmic model to study the transfer of norovirus during mechanical slicing of globe tomatoes. The model predicted that continuous slicing of approximately 100 tomatoes would cause a total accumulated transfer reduction of 4 logs of murine norovirus (Shieh et al., 2014)

Mechanical models have also been developed to investigate underlying principles affecting cross contamination during fresh produce processing (Zilelidou et al., 2015). For example, Zilelidou et al. (2015) used a semi-mechanical model to study microbial transfer between lettuce and knives. They concluded that this model could be used for risk assessment during leafy-green salad preparation (Zilelidou et al., 2015).

These previously mentioned models used for fresh produce processing can provide insightful information to aid retailers and processors in conducting risk assessment used to prepare fresh produce.

2.2 Cantaloupe

2.2.1 Cantaloupes and cantaloupe-associated foodborne illness

The cantaloupe, or muskmelon, is a warm-season annual plant that has an optimal temperature from 30 to 35°C. Typically, cantaloupes are cultivated on polyethylene mulch. Polyethylene mulch can increase soil temperature, provide frost protection, and inhibit weed growth in fields. Normally, cantaloupes are irrigated via dripping approach because a dripping system is portable and easy to manage (Hartz et al., 2008; Kemble, 1996). Most cantaloupe production in the United States are concentrated in California, Texas, and Arizona with cultivars such as Athena, Gold Rush, Sol Dorado, Sol Real, Mission, and Oro Rico (Kemble, 1996; Beaulieu, 2005). With an increasing demand for wholesome, fresh, and convenient food, cantaloupe has become a popular option among flavorful, nutritious fruits (Perkins-Veazie et al., 2012; USDA, 2018).

In recent years, cantaloupe has become one of the common food vehicles associated with foodborne illness. In 2006, a multistate outbreak of *Salmonella* Saintpaul in Australia caused 36 cases of illness (Munnoch et al., 2009). In 2011, contaminated cantaloupe from Jenson Farms caused one of the deadliest foodborne disease outbreaks in the United States, resulting in 147 cases of illness, 33 deaths, and 1 miscarriage across 28 states (CDC, 2012a). In 2012, one multistate outbreak of *Salmonella* Typhimurium and *Salmonella* Newport infections was linked to cantaloupe, which caused 3 deaths and 261 cases of illness (CDC, 2012b). In 2018, a multistate *Salmonella* Adelaide outbreak associated with pre-cut melon caused 77 cases of illness and 36 hospitalizations (CDC, 2018d).

2.2.2 Sources of pathogen contamination in cantaloupe supply chain

Numerous studies have investigated factors that may lead to pathogen contamination on cantaloupes in the supply chain (Ukuku et al., 2004; Ukuku, 2006; Ukuku & Sapers, 2001). An example of a cantaloupe supply chain is shown in Figure 2.1 (FDA, 2013). The potential sources

of cantaloupe contamination with foodborne pathogens are similar to the contamination sources of fresh produce supply chain as mentioned above.

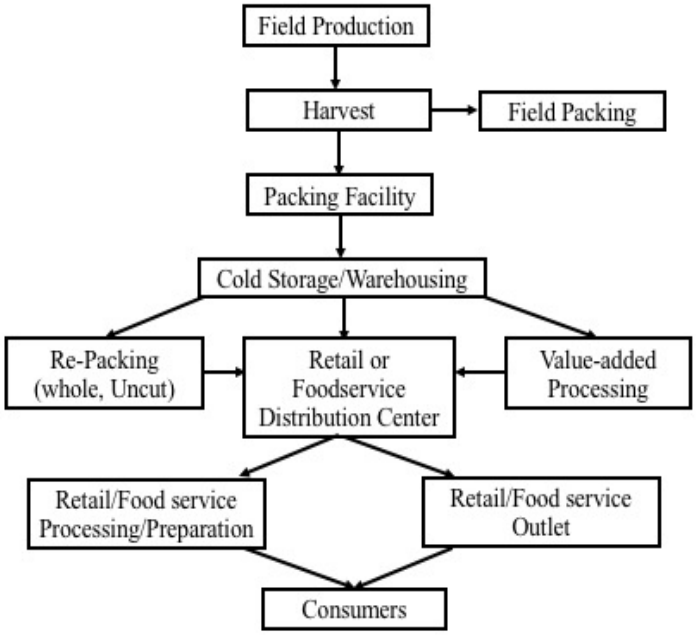

Figure 2.1 Supply chain flow for cantaloupe (Adapted from FDA, 2013)

Wounds and special structures, such as stem scars and netted rinds, of cantaloupes provide potential niches for bacterial attachment and survival during harvest and packing stages (FDA, 2013). These features increase the potential risks for cross contamination during cantaloupe processing (FDA, 2013).

Hydrocooling is an approach to extend the post-harvest life of fresh produce (Hartz et al., 2008). The typical hydrocooling practice uses cold water to dunk or wash the fresh produce (Erickson, 2012). Cantaloupe hydrocooling is a concern because of possible cross contamination (FAO/WHO, 2008). It was reported that a hydrocooler contaminated cantaloupe rinds with total

and fecal *enterococci* concentration up to 3.4 log CFU/g (Gagliardi et al., 2003). If the water used for chilling is contaminated, cantaloupe may be contaminated. Water containing foodborne pathogens is able to infiltrate stem scars or rind bruises and distribute within the fruit via the vascular system in hypodermal mesocarp (FDA, 2013; Macarisin et al., 2017).

Forced-air cooling is another method to remove the field heat from cantaloupes. It is an approach that exposes the packaged produce in a cooling room to higher air pressure on one side than the other (Suslow et al., 2000; Suslow et al., 2003). This method forces air through the packaged produce and removes the heat. However, if the forced air cooling equipment is not cleaned or sanitized properly, then the air may carry foodborne pathogens. If the air goes through the package of fresh cantaloupes, then the cantaloupes may be contaminated (Suslow et al., 2003).

After cantaloupes are shipped to retailers or caterers, they may be contaminated with foodborne pathogens by gloves and knives during processing. For example, Ukuku et al. (2001) concluded that fresh-cut cantaloupe pieces can be contaminated with *Salmonella* via knives.

2.2.3 Methods for the control of foodborne pathogens in cantaloupe supply chain

To decrease the amount of microorganisms on fresh cantaloupe, sanitizers such as chlorine are applied to the chilling water that is used for hydrocooling. Sanitizers may prevent the microbial build up in water. However, sanitizers cannot remove bacterial cells that have already lodged within the harborage sites of the produce (López-Gálvez et al., 2010; Erickson, 2012).

Chemical sanitizing treatment is a common practice to remove foodborne pathogens from the cantaloupe surface (Ukuku, 2006; Ukuku et al., 2004; Ukuku & Sapers, 2001). Ukuku et al. (2006) found that chlorine and hydrogen peroxide treatments greatly reduced bacterial

attachment on the cantaloupe surface. Other sanitizers, such as acidified sodium chlorite, acidified calcium sulfate, peroxyacetic acid, lactic acid, ozone, levulinic acid, and sodium dodecyl sulfate, have also greatly reduced microbial populations on cantaloupe (Fan et al., 2009; Vadlamudi et al., 2012; Webb et al., 2013). However, these sanitizers cannot effectively eliminate all pathogens present on cantaloupe (Fan et al., 2009; Vadlamudi et al., 2012; Webb et al., 2013).

Physical sanitation is also a common practice to remove foodborne pathogens from the produce surface. Brushing is a typical physical method (Erickson et al., 2015b). However, brushing is not able to remove all foodborne pathogens from the netted, porous rinds of cantaloupes, because the cantaloupe surface provides harborage sites for pathogens to evade the brush bristles (Erickson et al., 2015b).

Other methods for control of foodborne pathogens in the cantaloupe supply chain were developed. For example, Sangsuwan et al. (2008) found that chitosan/methyl cellulose film and vanillin film, the antimicrobial biodegradable films, can be used to wrap the fresh-cut cantaloupe and inhibit the growth of *E. coli* on fresh-cut cantaloupe.

2.3 *Listeria* and listeriosis

2.3.1 *Listeria* and public health

Listeria is a group of gram positive bacteria, which consists of 17 species. Among the genus *Listeria,* six species including *L. monocytogenes*, *L. ivanovii, L. seeligeri, L. innocua, L. welshimeri , and L. grayi* are genetically highly related (Bhunia, 2018). *L. monocytogenes* is pathogenic to humans (Bhunia, 2018). It is widespread and can be isolated from soil, decaying vegetation, silage, and animal intestines (Bhunia, 2018). *L. monocytogenes* is able to survive in

extreme conditions such as cold temperatures, low pH, and high salt concentration (Todd & Notermans, 2011; Soni et al., 2014).

Listeriosis causes a great public health and food safety concern due to its high mortality and morbidity (Bhunia, 2018). According to the CDC, it is estimated that 1,600 people get listeriosis and 260 die annually. Most infected people are immunocompromised such as pregnant women, newborns, elderly people, and people with weakened immune systems (CDC, 2018b).

2.3.2 Virulence determinants of *L. monocytogenes*

L. monocytogenes relies on the positive regulatory factor A (*prfA*) to achieve the switch from a saprophytic microorganism, that lives on dead or decomposing organic matter, to an intracellular pathogen. *PrfA* is regulated by an RNA thermosensor mechanism that can promote translation of prfA at 37°C (Freitag et al., 2009; Johansson et al., 2002). *PrfA* regulates a virulence cluster which is comprised of *prfA-plcA-hlyA-mpl-actA-plcB* and other virulence factors such as internalin genes (Bhunia, 2018). When *L. monocytogenes* infects the host, bacterial surface proteins InlA and InlB are responsible for cell invasion. InlA can bind to the receptor called E-cadherin on the epithelial cells and InlB can bind to the receptor called Met located on hepatocytes and endothelial cells. Once bacteria internalize into host cells, they can escape from the vacuole with the help of two phospholipases (PlcA and PlcB) and listeriolysin O which acidifies the phagosome membrane. Hpt can provide nutrition to bacteria for replication within the host cell cytosol. At the same time, mobility for cell to cell spread is acquired by Actin A in conjunction with actin in host cells (Freitag et al., 2009; Freitag et al., 1993; Vázquez-Boland et al., 2001). *L. monocytogenes* is not able to easily infect healthy people because of their effective immune response. However, *L. monocytogenes* may enter a systematic transmission

stage and infect immunocompromised people such as pregnant women or elderly people by fetal or central nervous systematic infections (Freitag et al., 2009).

2.3.3 *L. monocytogenes* in food processing facilities

L. monocytogenes can persist in food processing facilities for many years and even contaminate food (FDA, 2016). It has been reported that ready-to-eat (RTE) foods including dairy products, fresh salad, and deli hams can be associated with listerosis (FDA, 2016). Biofilms are one of the factors that results in food contamination with *L. monocytogenes* in food processing environments. Biofilms are cell communities which are composed of extracellular polymeric substances such as polysaccharides, proteins, lipids, or nucleic acids. Biofilms may be present on biotic (e.g. fresh produce surface) or abiotic surfaces (i.e. food contact surface) in food facilities (Poulsen, 1999; Watnick & Kolter, 2000). Biofilm formation is generally divided into three stages: 1) early attachment, 2) biofilm maturation, 3) detachment and return to the planktonic growth mode. At the stage of biofilm maturation, biofilms may increase the production of secondary metabolites and genetic communication, and form the protective environment which increases the resistance to sanitizers or UV light. The biofilm will release the planktonic cells to the environment at the third stage. This leads to the difficulties in eliminating the bacteria, which may cause potential risks to the food industry (O'Toole et al., 2000). To assess and identify biofilm formation, microtiter plate assays are commonly used to measure the amount of attached bacteria (Djordjevic et al., 2002; Salazar et al., 2013; Wassinger et al., 2013).

The ability of *L. monocytogenes* to survive in cold temperatures may contribute to *L. monocytogenes* persistence in the food processing environment. *L. monocytogenes* can survive in the cold temperature environment by altering its membrane lipid composition such as shortening the length of fatty acid chains to maintain the ideal fluidity required for proper enzyme activity

(Gandhi & Chikindas, 2007). *L. monocytogenes* can accumulate compatible solutes such as betaine and carnitine to stimulate its growth under cold stress. *L. monocytogenes* can produce cold shock proteins (Csps) and cold acclimation proteins (Caps) to balance the growth of bacteria at low temperatures. Meanwhile, the two proteins remove and repair the abnormal and damaged peptides caused by cold stress (Gandhi & Chikindas, 2007).

2.3.4 Regulatory Policy on *L. monocytogenes*

Currently, the FDA and other regulatory agencies in the U.S. have a zero-tolerance policy for *L. monocytogenes* in ready-to-eat food and cooked food. The sensitivity limit of detection methods is 0.04 organisms per gram of food (Shank et al., 1996). However, some other countries, such as Canada and Denmark, set a zero tolerance criterion for *L. monocytogenes* in foods that support the growth of *L. monocytogenes* with an extended shelf life (Nørrung, 2000). The debate among the countries is that complete absence of *L. monocytogenes* is unrealistic and limits the trade of food between countries because of the different regulations. Risk assessment data is still needed to set international standards of *L. monocytogenes* tolerance levels (Nørrung, 2000).

2.4 Transcriptomic analysis of foodborne pathogens

The transcriptome is the whole set of transcripts in a cell or an organism (Nagalakshmi et al., 2010). Four decades ago, a method called Northern blotting, used for detecting specific RNAs, became the earliest attempt to understand the cellular transcriptome (Alwine et al., 1977). Transcriptomic analysis was facilitated by the development of reverse transcription quantitative PCR (RT-qPCR) and microarray technology (Nagalakshmi et al., 2010). Nowadays, fast and high-throughput transcriptomic characterizations, such as whole transcriptome sequencing (RNA-seq), have been developed to investigate the cellular states, cellular activities, and

physiology of bacteria. RNA-seq is a major approach to characterize transcriptional profiles and study the impact of environmental conditions, stress factors, and chemical signals on gene expressions (Mutz et al., 2013).

In recent years, transcriptional analyses of foodborne pathogens on food matrices have been well documented (Deng et al., 2012; Fratamico et al., 2011; Kang et al., 2019; Tang et al., 2015). Fratamico et al. (2011) studied gene expression profiles of *E. coli* O157: H7 in ground beef extract (GBE) using a DNA microarray. They observed that up-regulated genes were involved in transcriptional regulation, acid shock protein expression, and polysaccharide biosynthesis. They also reported that down-regulated genes encoded energy metabolism proteins and protein degradation enzymes. Liu et al. (2008) studied physiological states of *L. monocytogenes* in skim milk based on its gene expression profile. They found that genes related to oligopeptide transport were induced, which indicated essential amino acids were required for bacterial adaptation. Tang et al. (2015) used RNA-seq to analyze the transcriptome of *L. monocytogenes* adaptation on vacuum-packaged cold smoked salmon. Gene ontology enrichment analysis showed that gene sets involved in cobalamin (Coenzyme B_{12}) biosynthesis, and ethanolamine and 1,2-propanediol utilization were up-regulated. These bioprocesses may facilitate *L. monocytogenes* adaptation on salmon. Since Tang et al. (2015) found that the major functional categories of up-regulated genes encoded a specific carbohydrate phosphotransferase system, they suggested that *L. monocytogenes* could utilize a broad range of carbohydrates on smoked salmon. A similar finding was found by Palumbo et al. (2005), who investigated *L. monocytogenes* attachment and growth on cut cabbage via reverse transcriptase PCR. They suggested that *L. monocytogenes* could use carbon sources from the environment because genes encoding sugar ABC transporters and mannose-specific phosphotransferase systems were

induced. However, Kang et al. (2019) used a custom-designed microarray to study the transcriptomic response of *L. monocytogenes* growth on cantaloupe and found a contradictory result. The result showed that the major functional categories for down-regulated genes were related to carbohydrate metabolism.

In addition, transcriptomic analysis of foodborne pathogens under stresses has also been studied. Deng et al. (2012) characterized the transcriptome of *Salmonella* Enteritidis under desiccation and starvation stresses in peanut oil, a low water activity matrix to simulate peanut butter. They found that *Salmonella* Enteritidis in peanut oil entered a physiologically dormant state featuring overall low levels of transcription, including the induction of few transcripts potentially important for bacterial survival and stress response. Ryan et al. (2015) investigated *Salmonella* survival in low-pH environments such as the stomach or low-pH produce. Results showed that energy metabolism, such as citric acid cycle and amino acid metabolism, was up-regulated to promote *Salmonella* survival in an acidic environment (Ryan et al., 2015). Other factors such as hyperosmotic and cold stress are also common in food processing facilities. Refrigeration can extend the shelf life of food. However, foodborne pathogens can survive and persist in low temperatures by altering their cellular responses (Allen et al., 2008; Durack et al., 2013). For example, genes in *L. monocytogenes* that are related to DNA maintenance, cell envelope modification, and cell division were up-regulated under hyperosmotic and cold stress (Durack et al., 2013). Carbohydrate metabolism and membrane transport of *L. monocytogenes* were suppressed under cold and hyperosmotic stress. (Durack et al., 2013). Hain et al. (2008) used transcriptomic analysis to study the σ^B regulon, an alternative sigma factor of *Listeria monocytogenes.* The regulon controls the general stress responses that contribute to bacterial survival under stressed conditions. Hain et al (2008) found that this regulon regulated solute

transporters, universal stress proteins, transcriptional regulators, osmoregulation factors, and virulence factors.

Transcriptomic analysis can inform a more detailed and fundamental understanding of bacterial physiology in food matrices. Based on the mechanisms of bacterial survival and persistence in different food systems, food processors may develop targeted preservation strategies to control pathogens on food (Liu & Ream, 2008). For example, a study of transcriptomic analysis of *L. monocytogenes* adaptation on meat with sodium lactate treatment showed that this treatment can inhibit flagella synthesis (Suo et al., 2018). In addition, Stasiewicz et al. (2011) studied the transcriptional response of *L. monocytogenes* adaptation on lactate and diacetate. Results suggested that the treatment of organic acids can interfere with bacterial energy generation and inhibit bacterial growth on food (Stasiewicz et al., 2011).

Transcriptomic analysis can provide gene targets for gene functional analysis. Wang et al. (2010) found that a putative stress regulatory gene, *ycfR*, was induced in *Salmonella enterica* Typhimurium and Enteritidis during chlorine treatment via transcriptomic analysis. Salazar et al. (2013) then studied gene functions via gene deletion and found that *ycfR*, and a biofilm formation gene, *sirA*, as well as a gene of previously unknown function, *yigG* contributed to *Salmonella* attachment on fresh produce. Additionally, Fink et al. (2012) identified genes in *E. coli* that were related to interaction, survival, and attachment to the lettuce leaf surface by using a microarray. Gene mutation analysis showed that genes involved in biofilm formation (*bhsA*) and curli production (*csgA*) were responsible for bacterial colonization. This study also showed that gene *csgA* was related to bacterial short term attachment (Fink et al., 2012).

Summary

Fresh produce has been frequently implicated as a vehicle for *L. monocytogenes* (CDC, 2018a). Consumption of raw fresh produce may lead to potential health risks (Todd & Notermans, 2011). Government agencies have set rules and guidelines to minimize the risk of *L. monocytogenes* contamination in fresh produce production. However, cross contamination of fresh produce with *L. monocytogenes* in produce supply chain still occurs (CDC, 2012a; CDC, 2015). One reason may be the persistence of *L. monocytogenes* in the food processing environment (FDA, 2016). Transcriptomic analysis enables a detailed assessment of physiological states of foodborne pathogens on food matrices (Tang et al., 2015). Also, transcriptomic profiling provides potential metabolic targets for effective control of *L. monocytogenes* on food (Kang et al., 2019).

References

Allen, K. J., Lepp, D., McKellar, R. C., & Griffiths, M. W. (2008). Examination of stress and virulence gene expression in *Escherichia coli* O157: H7 using targeted microarray analysis. *Foodborne Pathogens and Disease, 5*(4), 437-447.

Alwine, J. C., Kemp, D. J., & Stark, G. R. (1977). Method for detection of specific RNAs in agarose gels by transfer to diazobenzyloxymethyl-paper and hybridization with DNA probes. *Proceedings of the National Academy of Sciences, 74*(12), 5350-5354.

Baranyi, J., & Roberts, T. A. (1994). A dynamic approach to predicting bacterial growth in food. *International Journal of Food Microbiology, 23* (3-4), 277-294.

Beaulieu, J. C. (2005). Within-season volatile and quality differences in stored fresh-cut cantaloupe cultivars. *Journal of Agricultural and Food Chemistry, 53*(22), 8679-8687.

Bhunia, A. K. (2018). *Foodborne microbial pathogens: mechanisms and pathogenesis*: Springer.

Caldwell, K. N., Anderson, G. L., Williams, P. L., & Beuchat, L. R. (2003). Attraction of a free-living nematode, *Caenorhabditis elegans*, to foodborne pathogenic bacteria and its potential as a vector of *Salmonella* Poona for preharvest contamination of cantaloupe. *Journal of Food Protection, 66*(11), 1964-1971.

CDC (2012a). Multistate outbreak of listeriosis linked to whole cantaloupes from Jensen Farms, Colorado (final update). *Available at:* https://www.cdc.gov/listeria/outbreaks/cantaloupes-jensen-farms/index.html. *Accessed on August 14th 2018.*

CDC (2012b). Multistate outbreak of *Salmonella* Typhimurium and *Salmonella* Newport infections linked to cantaloupe (final update). *Available at:* https://www.cdc.gov/salmonella/typhimurium-cantaloupe-08-12/index.html

Accessed on November 14th 2018.

CDC (2015). Multistate outbreak of listeriosis linked to commercially produced, prepackaged caramel apples made from Bidart Bros. Apples (final update). *Available at: https://www.cdc.gov/listeria/outbreaks/caramel-apples-12-14/index.html Accessed on August 9th 2018.*

CDC (2018a). Foodborne outbreaks. *Available at:* https://www.cdc.gov/foodsafety/outbreaks/index.html . *Accessed on November 15th 2018.*

CDC (2018b). *Listeria* (Listeriosis). *Available at:* https://www.cdc.gov/listeria/ *Accessed on December 3rd 2018.*

CDC (2018c). Multistate outbreak of *E. coli* O157: H7 infections linked to romaine lettuce (final update). *Available at:* https://www.cdc.gov/ecoli/2018/o157h7-04-18/index.html *Accessed on November 16th 2018.*

CDC (2018d). Multistate outbreak of *Salmonella* Adelaide infections linked to pre-cut melon (final update). *Available at:* https://www.cdc.gov/salmonella/adelaide-06-18/index.html. *Accessed on November 14th 2018.*

Danyluk, M. D., Friedrich, L. M., & Schaffner, D. W. (2014). Modeling the growth of *Listeria monocytogenes* on cut cantaloupe, honeydew and watermelon. *Food Microbiology, 38*, 52-55.

Deng, X., Li, Z., & Zhang, W. (2012). Transcriptome sequencing of *Salmonella enterica* serovar Enteritidis under desiccation and starvation stress in peanut oil. *Food Microbiology, 30*(1), 311-315.

Dimitri, C., Tegene, A., & Kaufman, P. R. (2003). US fresh produce markets: marketing channels, trade practices, and retail pricing behavior. In: United States Department of Agriculture, Economic Research Service.

Djordjevic, D., Wiedmann, M., & McLandsborough, L. (2002). Microtiter plate assay for assessment of *Listeria monocytogenes* biofilm formation. *Applied and Environmental Microbiology, 68*(6), 2950-2958.

Durack, J., Ross, T., & Bowman, J. P. (2013). Characterisation of the transcriptomes of genetically diverse *Listeria monocytogenes* exposed to hyperosmotic and low temperature conditions reveal global stress-adaptation mechanisms. *PloS One, 8*(9), e73603.

Erickson, M. C. (2012). Internalization of fresh produce by foodborne pathogens. *Annual Review of Food Science and Technology, 3*, 283-310.

Erickson, M. C., Liao, J., Cannon, J. L., & Ortega, Y. R. (2015b). Role of brushes and peelers in removal of *Escherichia coli* O157: H7 and *Salmonella* from produce in domestic kitchens. *Journal of Food Protection, 78*(9), 1624-1631.

Erickson, M. C., Liao, J.-Y., Habteselassie, M. Y., & Cannon, J. L. (2018). Inactivation of *Escherichia coli* O157: H7 and *Salmonella* during washing of contaminated gloves in levulinic acid and sodium dodecyl sulfate solutions. *Food Microbiology, 73*, 275-281.

Fan, X., Annous, B. A., Keskinen, L. A., & Mattheis, J. P. (2009). Use of chemical sanitizers to reduce microbial populations and maintain quality of whole and fresh-cut cantaloupe. *Journal of Food Protection, 72*(12), 2453-2460.

FAO (2013). FAO Statistical Yearbook 2013. *Available at:* http://www.fao.org/docrep/018/i3107e/i3107e.PDF. *Accessed on November 15th 2018.*

FAO/WHO (2008). Microbiological hazards and melons. *Available at:* http://www.fao.org/tempref/AG/agn/jemra/Microbiological_hazards_and_Melons_Nov08.pdf. *Accessed on May 15th 2019.*

FDA (2008). Guidance for Industry: Guide to minimize microbial food safety hazards of fresh-cut fruits and vegetables. *Available at:* https://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/ProducePlantProducts/ucm064458.htm - ch10 *Accessed on December 17th 2018.*

FDA (2013). National commodity-specific food safety guidelines for cantaloupes and netted melons. *Available at:* https://www.fda.gov/downloads/Food/UCM365219.pdf *Accessed on November 14th 2018.*

FDA (2016). CPG Sec. 555.320 *Listeria monocytogenes*. *Available at:* https://www.fda.gov/iceci/compliancemanuals/compliancepolicyguidancemanual/ucm136694.htm. *Accessed on December 3rd, 2018*.

FDA (2017). Food Code. *Available at:* https://www.fda.gov/Food/GuidanceRegulation/RetailFoodProtection/FoodCode/ucm595139.htm *Accessed on November 16th 2018.*

FDA (2018a). FSMA final rule on produce safety. *Available at:* https://www.fda.gov/food/guidanceregulation/fsma/ucm334114.htm *Accessed on December 15th 2018.*

FDA (2018b). Raw manure under the FSMA final rule on produce safety. *Available at:* https://www.fda.gov/food/guidanceregulation/fsma/ucm482426.htm. *Accessed on March 21st 2019.*

Fink, R. C., Black, E., Hou, Z., Sugawara, M., Sadowsky, M. J., & Diez-Gonzalez, F. (2012). Transcriptional responses of *Escherichia coli* K12 and O157: H7 in association with lettuce leaves. *Applied and Environmental Microbiology*, 1752-1764.

Fratamico, P. M., Wang, S., Yan, X., Zhang, W., & Li, Y. (2011). Differential gene expression of *E. coli* O157: H7 in ground beef extract compared to tryptic soy broth. *Journal of Food Science, 76*(1), M79-M87.

Freitag, N. E., Port, G. C., & Miner, M. D. (2009). *Listeria monocytogenes*-from saprophyte to intracellular pathogen. *Nature Reviews Microbiology, 7*(9), 623-628.

Freitag, N. E., Rong, L., & Portnoy, D. A. (1993). Regulation of the prfA transcriptional activator of *Listeria monocytogenes*: multiple promoter elements contribute to intracellular growth and cell-to-cell spread. *Infection and Immunity, 61*(6), 2537-2544.

Gagliardi, J., Millner, P., Lester, G., & Ingram, D. (2003). On-farm and postharvest processing sources of bacterial contamination to melon rinds. *Journal of Food Protection, 66*(1), 82-87.

Gandhi, M., & Chikindas, M. L. (2007). *Listeria*: a foodborne pathogen that knows how to survive. *International Journal of Food Microbiology, 113*(1), 1-15.

Guillier, L. (2016). Predictive microbiology models and operational readiness. *Procedia Food Science, 7*, 133-136.

Hain, T., Hossain, H., Chatterjee, S. S., Machata, S., Volk, U., Wagner, S., Brors, B., Haas, S., Kuenne, C. T., & Billion, A. (2008). Temporal transcriptomic analysis of the *Listeria monocytogenes* EGD-e σ^B regulon. *BMC Microbiology, 8*(1), 20.

Hartz, T., Cantwell, M., Mickler, J., Mueller, S., Stoddard, S., & Turini, T. (2008). Cantaloupe production in California. *Available at:* https://anrcatalog.ucanr.edu/pdf/7218.pdf

Accessed on July 1st 2019

Jacxsens, L., Luning, P., Van der Vorst, J., Devlieghere, F., Leemans, R., & Uyttendaele, M. (2010). Simulation modelling and risk assessment as tools to identify the impact of climate change on microbiological food safety-The case study of fresh produce supply chain. *Food Research International, 43*(7), 1925-1935.

Jimenez, M., Siller, J. H., Valdez, J. B., Carrillo, A., & Chaidez, C. (2007). Bidirectional *Salmonella enterica* serovar Typhimurium transfer between bare/glove hands and green bell pepper and its interruption. *International Journal of Environmental Health Research, 17*(5), 381-388.

Johansson, J., Mandin, P., Renzoni, A., Chiaruttini, C., Springer, M., & Cossart, P. (2002). An RNA thermosensor controls expression of virulence genes in *Listeria monocytogenes*. *Cell, 110*(5), 551-561.

Johnston, L. M., Jaykus, L.-A., Moll, D., Martinez, M. C., Anciso, J., Mora, B., & Moe, C. L. (2005). A field study of the microbiological quality of fresh produce. *Journal of Food Protection, 68*(9), 1840-1847.

Kang, J., Burall, L., Mammel, M. K., & Datta, A. R. (2019). Global transcriptomic response of *Listeria monocytogenes* during growth on cantaloupe slices. *Food Microbiology, 77*, 192-201.

Kemble, J. M. (1996). Guide to the commercial production of muskmelon (cantaloupe) and related melons. *Publication ANR, 974*.

Liu, Y., & Ream, A. (2008). Gene expression profiling of *Listeria monocytogenes* strain F2365 during growth in ultrahigh-temperature-processed skim milk. *Applied and Environmental Microbiology, 74*(22), 6859-6866.

López-Gálvez, F., Gil, M. I., Truchado, P., Selma, M. V., & Allende, A. (2010). Cross-contamination of fresh-cut lettuce after a short-term exposure during pre-washing cannot be controlled after subsequent washing with chlorine dioxide or sodium hypochlorite. *Food Microbiology, 27*(2), 199-204.

Luo, Y., He, Q., & McEvoy, J. L. (2010). Effect of storage temperature and duration on the behavior of *Escherichia coli* O157: H7 on packaged fresh-cut salad containing romaine and iceberg lettuce. *Journal of Food Science, 75*(7), M390-M397.

Macarisin, D., Wooten, A., De Jesus, A., Hur, M., Bae, S., Patel, J., Evans, P., Brown, E., Hammack, T., & Chen, Y. (2017). Internalization of *Listeria monocytogenes* in cantaloupes during dump tank washing and hydrocooling. *International Journal of Food Microbiology, 257*, 165-175.

Materon, L. A., Martinez-Garcia, M., & McDonald, V. (2007). Identification of sources of microbial pathogens on cantaloupe rinds from pre-harvest to post-harvest operations. *World Journal of Microbiology and Biotechnology, 23*(9), 1281-1287.

Montville, R., Chen, Y., & Schaffner, D. W. (2001). Glove barriers to bacterial cross-contamination between hands to food. *Journal of Food Protection, 64*(6), 845-849.

Munnoch, S., Ward, K., Sheridan, S., Fitzsimmons, G., Shadbolt, C. T., Piispanen, J., Wang, Q., Ward, T., Worgan, T., & Oxenford, C. (2009). A multi-state outbreak of *Salmonella* Saintpaul in Australia associated with cantaloupe consumption. *Epidemiology & Infection, 137*(3), 367-374.

Mutz, K.-O., Heilkenbrinker, A., Lönne, M., Walter, J.-G., & Stahl, F. (2013). Transcriptome analysis using next-generation sequencing. *Current Opinion in Biotechnology, 24*(1), 22-30.

Nagalakshmi, U., Waern, K., & Snyder, M. (2010). RNA-Seq: a method for comprehensive transcriptome analysis. *Current Protocols in Molecular Biology, 89*(1), 11-14.

Nørrung, B. (2000). Microbiological criteria for *Listeria monocytogenes* in foods under special consideration of risk assessment approaches. *International Journal of Food Microbiology, 62*(3), 217-221.

O'Toole, G., Kaplan, H. B., & Kolter, R. (2000). Biofilm formation as microbial development. *Annual Reviews in Microbiology, 54*(1), 49-79.

Parnell TL, Harris LJ, Suslow TV. (2005). Reducing *Salmonella* on cantaloupes and honeydew melons using wash practices applicable to postharvest handling, foodservice, and consumer preparation. *International Journal Food Microbiology.* 99(1), 59-70.

Palumbo, J. D., Kaneko, A., Nguyen, K. D., & Gorski, L. (2005). Identification of genes induced in *Listeria monocytogenes* during growth and attachment to cut cabbage, using differential display. *Applied and Environmental Microbiology, 71*(9), 5236-5243.

Perkins-Veazie, P., Beaulieu, J. C., & Siddiq, M. (2012). Watermelon, cantaloupe and honeydew. *Tropical and Subtropical Fruits: Postharvest Physiology, Processing and Packaging. John Wiley & Sons, Inc*, 549-568.

Poulsen, L. V. (1999). Microbial biofilm in food processing. *LWT-Food Science and Technology, 32*(6), 321-326.

Ryan, D., Pati, N. B., Ojha, U. K., Padhi, C., Ray, S., Jaiswal, S., Singh, G. P., Mannala, G. K., Schultze, T., & Chakraborty, T. (2015). Global transcriptome and mutagenic analyses of the acid tolerance response of *Salmonella enterica* serovar Typhimurium. *Applied and Environmental Microbiology, 81*(23), 8054-8065.

Salazar, J. K., Deng, K., Tortorello, M. L., Brandl, M. T., Wang, H., & Zhang, W. (2013). Genes ycfR, sirA and yigG contribute to the surface attachment of *Salmonella enterica* Typhimurium and Saintpaul to fresh produce. *PloS One, 8*(2), e57272.

Salazar, J. K., Sahu, S. N., Hildebrandt, I. M., Zhang, L., Qi, Y., Liggans, G., Datta, A. R., & Tortorello, M. L. (2017). Growth kinetics of *Listeria monocytogenes* in cut produce. *Journal of Food Protection, 80*(8), 1328-1336.

Sangsuwan, J., Rattanapanone, N., & Rachtanapun, P. (2008). Effect of chitosan/methyl cellulose films on microbial and quality characteristics of fresh-cut cantaloupe and pineapple. *Postharvest Biology and Technology, 49*(3), 403-410.

Scollon, A. M., Wang, H., & Ryser, E. T. (2016). Transfer of *Listeria monocytogenes* during mechanical slicing of onions. *Food Control, 65*, 160-167.

Shank, F. R., Elliot, E. L., Wachsmuth, I. K., & Losikoff, M. E. (1996). US position on *Listeria monocytogenes* in foods. *Food Control, 7*(4-5), 229-234.

Shen, C., Luo, Y., Nou, X., Bauchan, G., Zhou, B., Wang, Q., & Millner, P. (2012). Enhanced inactivation of *Salmonella* and *Pseudomonas* biofilms on stainless steel by use of T-128, a fresh-produce washing aid, in chlorinated wash solutions. *Applied and Environmental Microbiology, 78*(19), 6789-6798.

Sheridan, G., Masters, C., Shallcross, J., & Mackey, B. (1998). Detection of mRNA by reverse transcription-PCR as an indicator of viability in *Escherichia coli* cells. *Applied and Environmental Microbiology, 64*(4), 1313-1318.

Shieh, Y. C., Tortorello, M. L., Fleischman, G. J., Li, D., & Schaffner, D. W. (2014). Tracking and modeling norovirus transmission during mechanical slicing of globe tomatoes. *International Journal of Food Microbiology, 180*, 13-18.

Soares, V. M., Pereira, J. G., Viana, C., Izidoro, T. B., dos Santos Bersot, L., & Pinto, J. P. d. A. N. (2012). Transfer of *Salmonella* Enteritidis to four types of surfaces after cleaning procedures and cross-contamination to tomatoes. *Food Microbiology, 30*(2), 453-456.

Soni, D. K., Singh, M., Singh, D. V., & Dubey, S. K. (2014). Virulence and genotypic characterization of *Listeria monocytogenes* isolated from vegetable and soil samples. *BMC Microbiology, 14*(1), 241.

Stasiewicz, M. J., Wiedmann, M., & Bergholz, T. M. (2011). The transcriptional response of *Listeria monocytogenes* during adaptation to lactate and diacetate includes synergistic changes that increase fermentative acetoin production. *Applied and Environmental Microbiology*, 5294-5306.

Suo, Y., Gao, S., Baranzoni, G. M., Xie, Y., & Liu, Y. (2018). Comparative transcriptome RNA-Seq analysis of *Listeria monocytogenes* with sodium lactate adaptation. *Food Control, 91*, 193-201.

Suslow, T. V., Cantwell, M., & Mitchell, J. (2000). Cantaloupe. *Recommendations for Maintaining Postharvest Quality. Product Facts. University of California: Davis, CA*.

Suslow, T., Oria, M., Beuchat, L., Garrett, E., Parish, M., Harris, L., Farber, J., & Busta, F. (2003). Production practices as risk factors in microbial food safety of fresh and fresh-cut produce. *Comprehensive Reviews in Food Science and Food Safety, 2*, 38-77.

Sy, K. V., Murray, M. B., Harrison, M. D., & Beuchat, L. R. (2005). Evaluation of gaseous chlorine dioxide as a sanitizer for killing *Salmonella*, *Escherichia coli* O157: H7, *Listeria monocytogenes*, and yeasts and molds on fresh and fresh-cut produce. *Journal of Food Protection, 68*(6), 1176-1187.

Tang, S., Orsi, R. H., den Bakker, H. C., Wiedmann, M., Boor, K. J., & Bergholz, T. M. (2015). Transcriptomic analysis of *Listeria monocytogenes* adaptation to growth on vacuum-packed cold smoked salmon. *Applied and Environmental Microbiology*, *81(19)*, 6812-6824.

Todd, E., & Notermans, S. (2011). Surveillance of listeriosis and its causative pathogen, *Listeria monocytogenes*. *Food Control, 22*(9), 1484-1490.

Ukuku, D. O. (2006). Effect of sanitizing treatments on removal of bacteria from cantaloupe surface, and re-contamination with *Salmonella*. *Food Microbiology, 23*(3), 289-293.

Ukuku, D. O., Pilizota, V., & Sapers, G. M. (2004). Effect of hot water and hydrogen peroxide treatments on survival of *Salmonella* and microbial quality of whole and fresh-cut cantaloupe. *Journal of Food Protection, 67*(3), 432-437.

Ukuku, D. O., & Sapers, G. M. (2001). Effect of sanitizer treatments on *Salmonella* Stanley attached to the surface of cantaloupe and cell transfer to fresh-cut tissues during cutting practices. *Journal of Food Protection, 64*(9), 1286-1291.

USDA (2018). National nutrient database for standard reference legacy release. *Available at:* https://ndb.nal.usda.gov/ndb/foods/show/301173?n1=%7BQv%3D1%7D&fgcd=&man=&lfacet=&count=&max=&sort=&qlookup=&offset=&format=Full&new=&measureby=&Qv=1&ds=&qt=&qp=&qa=&qn=&q=&ing= *Accessed on August 14th 2018.*

Vandamm, J. P., Li, D., Harris, L. J., Schaffner, D. W., & Danyluk, M. D. (2013). Fate of *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* on fresh-cut celery. *Food Microbiology*, *34* (1), 151-157.

Vadlamudi, S., Taylor, T. M., Blankenburg, C., & Castillo, A. (2012). Effect of chemical sanitizers on *Salmonella enterica* serovar Poona on the surface of cantaloupe and

pathogen contamination of internal tissues as a function of cutting procedure. *Journal of Food Protection, 75*(10), 1766-1773.

Van Boxstael, S., Habib, I., Jacxsens, L., De Vocht, M., Baert, L., Van de Perre, E., Rajkovic, A., Lopez-Galvez, F., Sampers, I., & Spanoghe, P. (2013). Food safety issues in fresh produce: bacterial pathogens, viruses and pesticide residues indicated as major concerns by stakeholders in the fresh produce chain. *Food Control, 32*(1), 190-197.

Van Vliet, A. H. (2010). Next generation sequencing of microbial transcriptomes: challenges and opportunities. *FEMS Microbiology Letters, 302*(1), 1-7.

Vázquez-Boland, J. A., Kuhn, M., Berche, P., Chakraborty, T., Domínguez-Bernal, G., Goebel, W., González-Zorn, B., Wehland, J., & Kreft, J. (2001). *Listeria* pathogenesis and molecular virulence determinants. *Clinical Microbiology Reviews, 14*(3), 584-640.

Wachtel, M. R., McEVOY, J. L., Luo, Y., Williams-Campbell, A. M., & Solomon, M. B. (2003). Cross-contamination of lettuce (*Lactuca sativa* L.) with *Escherichia coli* O157: H7 via contaminated ground beef. *Journal of Food Protection, 66*(7), 1176-1183.

Wang, S., Phillippy, A. M., Deng, K., Rui, X., Li, Z., Tortorello, M. L., & Zhang, W. (2010). Transcriptomic responses of *Salmonella enterica* serovars Enteritidis and Typhimurium to chlorine-based oxidative stress. *Applied and Environmental Microbiology, 76*(15), 5013-5024.

Wassinger, A., Zhang, L., Tracy, E., Munson Jr, R. S., Kathariou, S., & Wang, H. H. (2013). Role of a GntR-family response regulator LbrA in *Listeria monocytogenes* biofilm formation. *PloS One, 8*(7), e70448.

Watnick, P., & Kolter, R. (2000). Biofilm, city of microbes. *Journal of Bacteriology, 182*(10), 2675-2679.

Webb, C. C., Davey, L. E., Erickson, M. C., & Doyle, M. P. (2013). Evaluation of levulinic acid and sodium dodecyl sulfate as a sanitizer for use in processing Georgia-grown cantaloupes. *Journal of Food Protection, 76*(10), 1767-1772.

Wu, J., & Ponder, M. A. (2018). Evaluation of transfer rates of *Salmonella* from single-use gloves and sleeves to dehydrated pork jerky. *Food Control, 84*, 17-22.

Zilelidou, E. A., Tsourou, V., Poimenidou, S., Loukou, A., & Skandamis, P. N. (2015). Modeling transfer of *Escherichia coli* O157: H7 and *Listeria monocytogenes* during preparation of fresh-cut salads: impact of cutting and shredding practices. *Food Microbiology, 45*, 254-265.

Zwietering, M. H., & den Besten, H. M. (2011). Modelling: One word for many activities and uses. *Food Microbiology, 28*(4), 818-822.

CHAPTER 3

GLOVE-MEDIATED TRANSFER OF *LISTERIA MONOCYTOGENES* TO FRESH-CUT CANTALOUPE UNDER LABORATORY CONDITIONS[1]

[1] Qi Y and Deng X. Submitted to *Food Microbiology*

ABSTRACT

The common use of gloves in retail practices represents a potential route for cross contamination of foodborne pathogens in fresh-cut produce. Using fresh-cut cantaloupe as a food model, we investigated factors that may influence glove-mediated cross contamination by *Listeria monocytogenes* and developed mathematical models to illustrate the patterns of transfer during fresh-cutting practices. Cantaloupe pieces (rind, stem scar tissue, or flesh) were spot inoculated with a mixture of four *L. monocytogenes* strains. A gloved hand was first placed in contact with a contaminated cantaloupe sample, followed by contacting uncontaminated fresh-cut cantaloupe samples. Contact time (2, 5, 10 s), contact pressure (0.05, 0.18, 0.37 psi), and glove type (nitrile, polyvinyl chloride, polyethylene) did not have a significant effect on transfer of *L. monocytogenes* from cantaloupe rind to flesh, or from flesh to flesh. However, glove type appeared to affect *L. monocytogenes* transfer from the stem scar tissue to cantaloupe flesh (P = 0.0371). Transfer from rind pieces that had been washed with water was significantly higher than transfer from pieces that had not been washed (P = 0.0006). Predictive modeling and experimental validation showed that *L. monocytogenes* transfer occurs on pieces of cantaloupe flesh sequentially touched 85 times by a gloved hand. Findings of the study provide new scientific data to aid researchers, retailers, and caterers in safety risk assessments of fresh-cut practices used to prepare cantaloupes and other produce items.

Key words: *Listeria monocytogenes*, transfer, cross contamination, gloves, cantaloupe, modeling

3.1 Introduction

A number of recent foodborne outbreaks of listeriosis in the United States have been linked to fresh and fresh-cut produce (Garner & Kathariou, 2016). Infections linked to celery (Gaul et al., 2013), cantaloupe (CDC, 2012), stone fruit (Jackson et al., 2015), and caramel apples (CDC, 2015) have been documented. Consumer knowledge that minimally processed produce is a vital part of a healthy diet has resulted in its demand as a wholesome, fresh, and convenient food (Rico et al., 2007). Cantaloupe is a popular fresh-cut fruit because of its sensory quality and nutritional value (Bett-Harber et al., 2011; Perkins-Veazie et al., 2012; USDA, 2018). In recent years, cantaloupe has emerged as a reoccurring vehicle in several human foodborne disease outbreaks of listeriosis. For instance, in 2011, consumption of cantaloupe contaminated with *L. monocytogenes* resulted in 147 cases of illness, 33 deaths, and 1 miscarriage across 28 states in the U.S. (CDC, 2012). In 2018, an outbreak with at least 22 confirmed cases of human listeriosis was linked to contaminated cantaloupes in Australia (NSW, 2018).

Outbreaks of listeriosis linked to cantaloupes prompted our research interest to investigate factors that may lead to contamination in fresh-cut cantaloupe and other produce items. The unique porous, netted rind structure of several popular varieties of cantaloupes provides a suitable surface for bacterial attachment and biofilm formation, which may subsequently lead to cross contamination of the edible portion during minimal processing (Annous et al., 2005;Perkins-Veazie et al., 2012; Ukuku and Fett, 2006). Wash water containing pathogens can facilitate infiltration of pathogens into cantaloupes via stem scar tissue and contamination of the flesh through the vascular system in the hypodermal mesocarp (Macarisin et al., 2017). Critical conditions such as washing and cutting practices, storage temperature, and

personnel hygiene in the cantaloupe supply chain should also be considered when applying interventions to minimize microbial cross contamination (Parnell et al., 2003; Perkins-Veazie et al., 2012).

Minimal processing of fresh-cut produce at retailers typically includes the following steps: (1) storage at a temperature-controlled distribution center, (2) washing, (3) chilling, (4) fresh-cutting (e.g., peeling and dicing), and (5) display and sale. Stainless steel knives and mechanical slicers as well as gloved hands are frequently used for preparing fresh-cut produce, and are potential vehicles for microbial cross contamination to finished products (Jimenez et al., 2007; Shieh et al., 2014; Erickson et al., 2015). For example, Erickson et al. (2015) demonstrated the role of knives in cross contaminating sliced tomatoes, cucumbers, strawberries, cantaloupes, and honeydew melons with *Escherichia coli* O157:H7 and *Salmonella*. They showed that average contamination by the two pathogens ranged from 3 to 43%. Jimenez et al. (2007) reported that the percentages of *Salmonella* Typhimurium transfer from green bell peppers to gloved hands and gloved hands to green bell peppers were 46.6% and 0.84%, respectively.

Several mathematical models have been developed for studying transfer of foodborne pathogens in various food commodities. Exponential decay models were used to study *L. monocytogenes* transfer in onions and salami (Scollon et al., 2016; Sheen, 2008); logarithmic and power models were used for norovirus transfer in tomatoes (Shieh et al., 2014). Mechanical models have also been developed to investigate the role of food-contact surfaces in bacterial cross contamination during processing (Zilelidou et al., 2015). For example, Møller et al. (2012) evaluated the transfer of *Salmonella enterica* from the grinder to ground pork using a semi-mechanical model. This model was also used for predicting microbial transfer between lettuce and knives (Zilelidou et al., 2015).

Some previous studies reported that cutting utensils play an important role in cross contamination of *L. monocytogenes* in fresh-cut produce (Scollon et al., 2016; Ukuku and Fett, 2002; Zilelidou et al., 2015). However, research data on *L. monocytogenes* transfer behavior via gloves to fresh-cut produce are scarce. To fill this knowledge gap, in this study, we used fresh-cut cantaloupe as a food model to investigate *L. monocytogenes* transfer by gloves. Possible impacts of factors such as contact time, contact pressure, and glove type in the glove-mediated transfer behavior of *L. monocytogenes* were evaluated. Mathematical models that illustrate transfer of *L. monocytogenes* via gloves on cantaloupe pieces were developed.

3.2 Materials and methods

3.2.1 Strains used and preparation of inoculum

An inoculum containing four strains of *L. monocytogenes* from our culture collection at the Center for Food Safety, University of Georgia was prepared: strain F8027 (serovar 4b, a celery isolate), F8369 (serovar 1/2a, a corn isolate), F8255 (serovar 1/2b, a peach isolate), and ATCC 51779 (serovar 1/2c, a cheese isolate). These strains represent serotypes most frequently associated with foodborne listeriosis (Doumith et al., 2004; Doumith et al., 2005; Ryser and Marth, 1999). Frozen stock cultures (-20°C) were thawed, transferred (10 µl) into 5 ml of tryptic soy broth (TSB; Becton Dickinson, Sparks, MD) supplemented with 0.6% (wt/vol) yeast extract (Becton Dickinson) (TSB-YE), and incubated with shaking (200 rpm) for 18 - 20 h at 37°C. Cultures were streaked on tryptic soy agar (TSA; Becton Dickinson) supplemented with 0.6% (wt/vol) yeast extract (TSA-YE) and incubated at 37°C for 24 h. Cells from a single colony of each strain were transferred to 5 ml of TSB-YE and incubated with shaking (200 rpm) at 37°C for 16 - 18 h. Cultures were adjusted to an optical density at 600 nm (OD_{600}) of 0.8 and washed in 1× phosphate buffered saline (PBS; AccuGENE, Rockland, ME). Cell suspensions of each

strain were combined to make a mixture of strains containing 8 - 9 log CFU/ml, followed by serially diluting to give a suspension containing 6 - 7 log CFU/ml (high inoculum, HI) and 4 - 5 log CFU/ml (low inoculum, LI). These suspensions were used to inoculate fresh-cut cantaloupe rind, stem scar tissue, and flesh.

3.2.2. Preparation and inoculation of cantaloupe

Cantaloupes (*Cucumis melo* L. var. reticulatus) were purchased at two retail supermarkets in Griffin, Georgia, and stored at 4°C for at least 24 h before use. Cantaloupes were thoroughly washed with tap water, dried, and cut with a sterile stainless steel knife on a sterile plastic cutting board. Rind, stem scar tissue, and flesh were cut into rectangular pieces (4.5 × 3.5 × 0.7 cm, 12±1g /piece). Pieces with stem scar tissue also included a portion of the rind. The external surface of rind and stem scar pieces, as well as flesh pieces (4.5 cm × 3.5 cm area), were inoculated (100 µl/piece of HI or LI). Inocula were evenly spread over the surface areas of rind, scar tissue area on pieces containing the stem scar, and flesh. For rind and stem scar pieces, inoculated and uninoculated negative control samples were stored at 4°C for 16 - 18 h prior to glove-mediated transfer experiments. Flesh samples were used within 1 h after cutting. Sample preparation and inoculation was conducted at 10°C to simulate an in-store processing environment.

3.2.3 Glove-mediated transfer of *L. monocytogenes*

To study glove-mediated transfer of *L. monocytogenes* in fresh-cut cantaloupe, we evaluated three factors (i.e., glove contact time, contact pressure, and glove type). Three contact times (2, 5, and 10 s), three contact pressures (0.05, 0.18, and 0.37 psi), and three types of gloves (nitrile by Microflex, Reno, NV, polyethylene by Ansell Healthcare, Red Bank, NJ, and polyvinyl chloride gloves by Ambitex, Cleveland, OH) were compared.

To compare different contact times, a nitrile glove was used by first pressing on a piece of uninoculated flesh. The palm of the gloved hand then pressed the surface of an inoculated flesh sample (LI or HI), followed by consecutively pressing five (LI) or nine (HI) pieces of uninoculated cantaloupe flesh. A pressure of approximately 0.18 psi was used for each contact.

To compare different contact pressures, two additional contact pressures created by weights (0.05 and 0.37 psi) were compared to hand pressing (0.18 psi) for 10 s. The hand pressing pressure was determined using a scale. A hand fitted with a nitrile glove as well as nitrile gloves pressured by weights were separately placed in contact with an uninoculated piece of cantaloupe flesh, followed by contact with a piece of inoculated flesh (LI or HI) for 10 s. Then the palm of the gloved hand as well as weight-pressured gloves were used to consecutively press five (LI) or nine (HI) pieces of uninoculated cantaloupe flesh.

To compare different glove types, we used a pressure of 0.18 psi and a contact time of 10 s. The three types of gloves were individually used to press a piece of uninoculated cantaloupe flesh, followed by placing on cantaloupe inoculated with LI or HI. The palm of gloved hands was used to sequentially press 25 (LI) or 40 (HI) samples of uninoculated cantaloupe flesh. Data obtained from sequentially pressing pieces of cantaloupe flesh were used for predictive modeling as described in Section 3.2.5.

The transfer of *L. monocytogenes* from rind and stem scar tissue to cantaloupe flesh was investigated in a manner similar to that used in flesh to flesh transfer studies. Briefly, pieces of rind with and without stems scar tissue were immersed in 50 ml of sterile water for 5 min, and placed on Petri dish lids to enable surface water to evaporate for 5 min at 10°C. The palm of a gloved hand was placed on an inoculated (HI) cantaloupe rind or stem scar, followed by pressing an uninoculated cantaloupe flesh sample.

Lastly, the effect of moisture on the surface of rind and stem scar pieces on transfer of *L. monocytogenes* to cantaloupe flesh via gloved hands was evaluated. Inoculated rind and stem scar pieces, with and without washing with water, were compared. A nitrile gloved hand was placed in contact with a rind or stem scar piece (HI) for 10 s, then, using the same pressure (0.18 psi) placed in contact with a flesh piece for 10 s.

3.2.4 Enumeration of *L. monocytogenes*

Each inoculated or uninoculated piece of cantaloupe (12±1 g of rind, stem scar, or flesh) was placed in a sterile stomacher bag containing 60 ml of buffered peptone water (BPW, Becton Dickinson). Samples were hand massaged for 60 s and pummeled in a stomacher (Stomacher model 400, Seward Ltd., Westberry, NY) at 230 rpm for 60 s. The homogenate was serially diluted in BPW, surface plated on Oxford Listeria agar base (Neogen, Lansing, MI) supplemented with modified Oxford *Listeria* supplement (20 mg/L moxalactam, 10 mg/L colistin sulfate) (Dalynn, Calgary, Alberta, CA) (MOX), and incubated for 48 h at 37°C. The cantaloupe/BPW homogenate was combined with 72 ml of 2× *Listeria* enrichment broth (LEB, Neogen) and incubated for 48 h at 30°C. When *L. monocytogenes* was not detected by direct plating on MOX (<1.86 log CFU/piece), the enriched homogenate was streaked on MOX and incubated at 37°C for 48 h. Representative colonies were subjected to *L. monocytogenes* confirmation by PCR analysis using primers that target a 2,046-bp segment of the *L. monocytogenes*-specific *iap* gene (GenBank accession no.X52268). The forward primer sequence was 5'- AACTGGTTTCGTTAACGGTAAATACTTA-3' and the reverse primer was 5'- TAGGCGCAGGTGTAGTTGCT-3' (FDA, 2018). Populations of *L. monocytogenes* determined by plating are expressed as CFU/piece.

3.2.5 Model development and validation

3.2.5.1 Model development

TableCurve2D V5.0 (SYSTAT Software Inc., Richmond, CA) software was used to develop mathematical models for glove-mediated transfer of *L. monocytogenes* on cantaloupe flesh. Models with four features were favored: (1) higher correlation coefficient values (R^2) between fitted values and observed values, (2) *L. monocytogenes* counts tailing off and approaching zero after extended consecutive transfer, (3) fewer parameters, and (4) no singularity and divergence (Sheen, 2008). Three mathematical models chosen to fit the transfer data were logarithmic (Eq[1]), power (Eq[2]) , and exponential decay (Eq[3]) :

$Y = a_1 + b_1 \cdot \ln x$ [1]

$Y = a_2 \cdot x^{b2}$ [2]

$Y = a_3 \cdot e^{(-x/b3)}$ [3]

$\text{NRMSE} = \frac{RMSE}{Y_{max} - Y_{min}}$ [4]

where Y is the number of *L. monocytogenes* transferred (log CFU/piece), x is the number of fresh-cut cantaloupe pieces (flesh) pressed, and a_1, a_2, a_3, b_1, b_2, and b_3 are regression parameters. R^2 was used to evaluate model performance. NRMSE is the normalized root mean squared error (Eq[4]), RMSE is the root mean squared error, which measures the average deviation between observed and fitted values, and Y_{max} and Y_{min} are the maximum and the minimum values taken by Y. NRMSE values approaching zero indicate a closer fit with observed data. All three models have been used in previous microbial transfer studies on food (Aarnisalo et al., 2007; Scollon et al., 2016; Shieh et al., 2014).

3.2.5.2 Model validation

Continuous transfer of *L. monocytogenes* via gloved hand was validated. Three volunteers were recruited to participate in transfer trials. Each volunteer pressed an uninoculated

piece of cantaloupe flesh with 0.18 psi pressure for 10 s with a nitrile gloved hand before pressing an inoculated piece of flesh (HI, 5 - 6 log CFU/piece). Volunteers then consecutively pressed up to 114 pieces with the palm of same gloved hand while maintaining the same contact time (10 s) and pressure (0.18 psi) for each press. The uninoculated piece and the 85th, 99th, and the 115th pieces were separately combined with 60 ml of BPW, hand massaged for 60 s, and pummeled in a stomacher (Stomacher model 400) at 230 rpm for 60 s. Homogenates were subjected to enrichment and PCR-based confirmation for the presence of *L. monocytogenes* as described above. Five replicate trials were conducted.

To evaluate performance of the model, a set of data that was not used to develop models was applied. Linear regression analysis of the experimental value and the value predicted by the logarithmic model was done (Bialka et al., 2008).

3.2.6 Statistical analysis

All transfer experiments were independently repeated on at least three different days (biological replicates), each with two samples (technical replicates). Statistical analysis was performed on a minimum of six data points for each treatment using TableCurve 2D for modeling and JMP Pro 14 (SAS Institute Inc., Cary, NC) for one-way ANOVA. Tukey's honest significance difference (HSD) test was used to identify significant differences mean values ($P \leq 0.05$).

3.3 Results and discussion

3.3.1 Glove-mediated transfer of *L. monocytogenes*

Statistical analysis showed that different glove types (i.e., nitrile, polyethylene, and polyvinyl chloride gloves), contact times (i.e., 2, 5, and 10 s), and contact pressures (i.e., 0.05, 0.18, and 0.37 psi) did not have a significant effect ($P > 0.05$) on the transfer of *L.*

monocytogenes on fresh-cut cantaloupe flesh (Figure 3.1). In contrast, other factors such as surface moisture, have been reported to promote bacterial transfer (Patrick et al., 1997). We propose that due to the high moisture nature of fresh-cut cantaloupe surface, the relatively loose attachment of *L. monocytogenes* may facilitate transfer via gloves and consequently minimize the potential impacts of other conditions.

The average transfer rate of *L. monocytogenes* on cantaloupe flesh pieces with an initial low inoculum was 4.16%, in contrast to 0.42% on high-inoculum pieces after six sequential transfers. The transfer rate was calculated based on the number of CFU on the sixth piece divided by the number of CFU on the initial contaminated piece (inoculum) × 100 (Montville & Schaffner, 2003). This finding shows that a higher transfer rate of *L. monocytogenes* when the initial number of *L. monocytogenes* on the flesh piece was low. This observation is consistent with other studies which demonstrated that bacterial transfer rate is inversely correlated with the population on the surface of the source (Montville and Schaffner, 2003). Pérez-Rodríguez et al. (2007) found that a lower inoculum led to a higher transfer coefficient for *Staphylococcus aureus* during slicing of a cooked meat product. Fravalo et al. (2009) showed that transfer of *Campylobacter* from poultry legs to cutting boards is inversely related to the initial number of bacteria present on poultry skins.

In our study, the transfer of *L. monocytogenes* from rind and stem scar pieces to flesh pieces was low with a low initial inoculum (data not shown). For this reason, subsequent studies and analysis focused on data collected with high inoculum. The glove type did not show a significant effect on the number of *L. monocytogenes* transferred from rind to cantaloupe flesh; however, there was a significant effect ($P \leq 0.05$) on transfer from stem scar tissue to cantaloupe flesh ($P = 0.0371$) (Fig 3.2.A). Contact time and contact pressure did not have a significant effect

($P > 0.05$) on the transfer of *L. monocytogenes* from the surface of cantaloupe rind or stem scar to cantaloupe flesh (Figure 3.2.B and Figure 3.2.C, respectively). Wash treatment did not significantly affect ($P > 0.05$) the initial number of *L. monocytogenes* on cantaloupe rind or stem scar surface (data not shown). Interestingly though, in the presence of residual moisture on rind pieces, gloves transferred a significantly higher number of *L. monocytogenes* to cantaloupe flesh pieces ($P = 0.0006$) (Figure 3.2.D). Residual water on stem scar pieces did not result in a significant difference in the number of *L. monocytogenes* transferred to cantaloupe flesh (Figure 3.2.D). These findings are in agreement with a previous study reported by Webb et al. (2013) which suggested that the reduction of *Salmonella* Poona on stem scars of cantaloupe was less than that on rinds treated with sanitizer or water. This could be partially due to the high porosity of stem scar tissue which enables bacterial trapping in cantaloupe tissues (Richards & Beuchat, 2004; Webb et al., 2013). Several studies have shown that the produce surface moisture adversely affects the attachment of *Salmonella* and therefore results in higher transfer from rind to flesh. Moisture did not influence transfer of *L. monocytogenes* from the stem scar to flesh because the pathogen may have infiltrated the porous tissue, thereby avoiding direct contact with and transfer to glove. In current processing practices, washing cantaloupes is a common practice. However, the time needed to completely dry washed cantaloupes and minimize bacterial transfer is not well understood.

3.3.2 Model development and validation

3.3.2.1 Model development

We used quantitative transfer data collected from experiments using a high inoculum to develop predictive models. The R^2 and NRMSE values were > 0.72 and < 0.16, respectively, in all three decay models, which indicates a good fit of the experimental data (Table 3.1, Figure

3.3). Data collected from experiments using a lower inoculum of *L. monocytogenes* did not fit well ($R^2 < 0.61$ and NRMSE was 0.17 - 0.22) (Table 3.1, Figure 3.5). Similar studies done by others have shown that low inoculation levels make predictive modeling difficult. For instance, Sheen et al. (2008) reported that low inocula at 3 or 4 log CFU/slice of salami made surface transfer modeling challenging. Scollon et al. (2016) showed that the model they used to fit the data on transfer of *L. monocytogenes* to onions via a slicer was less accurate with a low initial inoculum. Decay models have been used to study slicer- and knife-mediated bacterial transfer on foods (Aarnisalo et al., 2007; Scollon et al., 2016; Sheen 2008; Shieh et al., 2014); however, there are very few studies focused on developing models for transfer of foodborne pathogens to foods via gloves (Ivanek et al., 2004; Xiao et al., 2018). In our study, a rapid decay of bacterial transfer was observed on cantaloupe flesh pieces initially containing a high inoculum. A similar transfer pattern has been observed in knife- or slicer-mediated transfer of norovirus on globe tomatoes (Shieh et al., 2014), *L. monocytogenes* on onions (Scollon et al., 2016), and *L. monocytogenes* on salami (Sheen, 2008). These studies used logarithmic, exponential decay, or power models to predict pathogen transfer.

As suggested by the highest R^2 value and lowest NRMSE value for nitrile gloves and polyvinyl chloride gloves (Table 3.1), the logarithmic model ($Y = a_1 + b_1 \cdot \ln x$) outperformed the power model ($Y = a_2 \cdot x^{b2}$) and the exponential decay model ($Y = a_3 \cdot e^{(-x/b3)}$) in predicting *L. monocytogenes* transfer from a high inoculum. The power model showed a higher R^2 value and a lower NRMSE value for polyethylene gloves compared with other models. Compared to the asymptotic tail of the power model, the logarithmic model indicated that *L. monocytogenes* transfer from highly contaminated cantaloupe flesh pieces (high inoculum) would cease at the 99^{th} , 94^{th} , and 116^{th} piece (log CFU/piece = 0) through sequential pressing via nitrile, polyvinyl

chloride and polyethylene gloves, respectively. These estimates were based on the model parameters (a_1 and b_1) shown in Table 3.2.

3.3.2.2 Model validation

Models for transfer of *L. monocytogenes* from cantaloupe flesh pieces initially containing a low inoculum to uninoculated pieces were not an accurate prediction of transfer. However, the logarithmic model ($Y = a_1 + b_1 \cdot \ln x$) for cantaloupe pieces initially containing a high inoculum appeared to be a good fit with observed data for all three types of gloves (Table 3.1). This model was validated by additional experiments simulating extended sequential transfer of *L. monocytogenes* on cantaloupe flesh pieces via gloved hands. There was a good correlation between the predicted value and experimental results ($R^2 = 0.91065$) (Figure 3.4). The logarithmic model predicted transfer of *L. monocytogenes* can reach up to 99 pieces of flesh by nitrile glove contact. Our experimental validation showed that transfer of the pathogen dropped below the detection limit by culture enrichment (1 CFU/piece) between the 85^{th} and 99^{th} pieces, which is consistent with the model prediction. Both the observed and predicted values are in agreement with a report by Chen et al. (2014) which demonstrated that transfer of foodborne pathogens can occur for up to 100 slices of deli meat during slicing.

3.4 Conclusions

Our study is not without some limitations. For example, fresh-cut cantaloupe pieces were cut into a rectangular shape, and we used the palm of a hand to press fresh-cut cantaloupe pieces. These practices may not always reflect actual cutting practice. Also, the size and the shape of cantaloupe stem scar tissue vary among cantaloupe varieties and cantaloupe harvest practices. On the other hand, we used two inoculation levels (6 - 7 log CFU/ml and 4 - 5 log CFU/ml) of *L. monocytogenes*. These inocula may be high in terms of realistic contamination levels in fresh-cut

cantaloupes. However, factors affecting transfer of *L. monocytogenes* on cantaloupe pieces have been clearly demonstrated.

The U.S. Food and Drug Administration (FDA) Food Code (FDA, 2017) recommends that food handlers in retail settings avoid direct contact with ready-to-eat food. Single-use gloves are commonly used as a physical barrier to ensure food hygiene and avoid food contamination. In our study, we demonstrated that single-use gloves can serve as a vehicle for *L. monocytogenes* transfer between contaminated and uncontaminated fresh-cut cantaloupe. We further demonstrated that transfer is independent of different glove types. In a scenario where some cantaloupes are contaminated with *L. monocytogenes*, frequent changing of single-use gloves during cutting cantaloupes would likely reduce the risk of cross contamination with *L. monocytogenes*. Other than the glove type, other factors such as the food type may also affect microbial transfer. For example, Dickson (1990) reported higher transfer rates of *L. monocytogenes* and *Salmonella* Typhimurium from lean beef tissue compared with fat tissue. Additional studies focusing on different aspects of food processing and handling will be necessary to better understand factors affecting cross contamination of food commodities with foodborne pathogens and to validate predictive models.

3.5 Acknowledgements

This study was supported by the Agriculture and Food Research Initiative (AFRI) awards no.2016-67017-24432 from the U.S. Department of Agriculture (USDA) National Institute of Food and Agriculture (NIFA). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

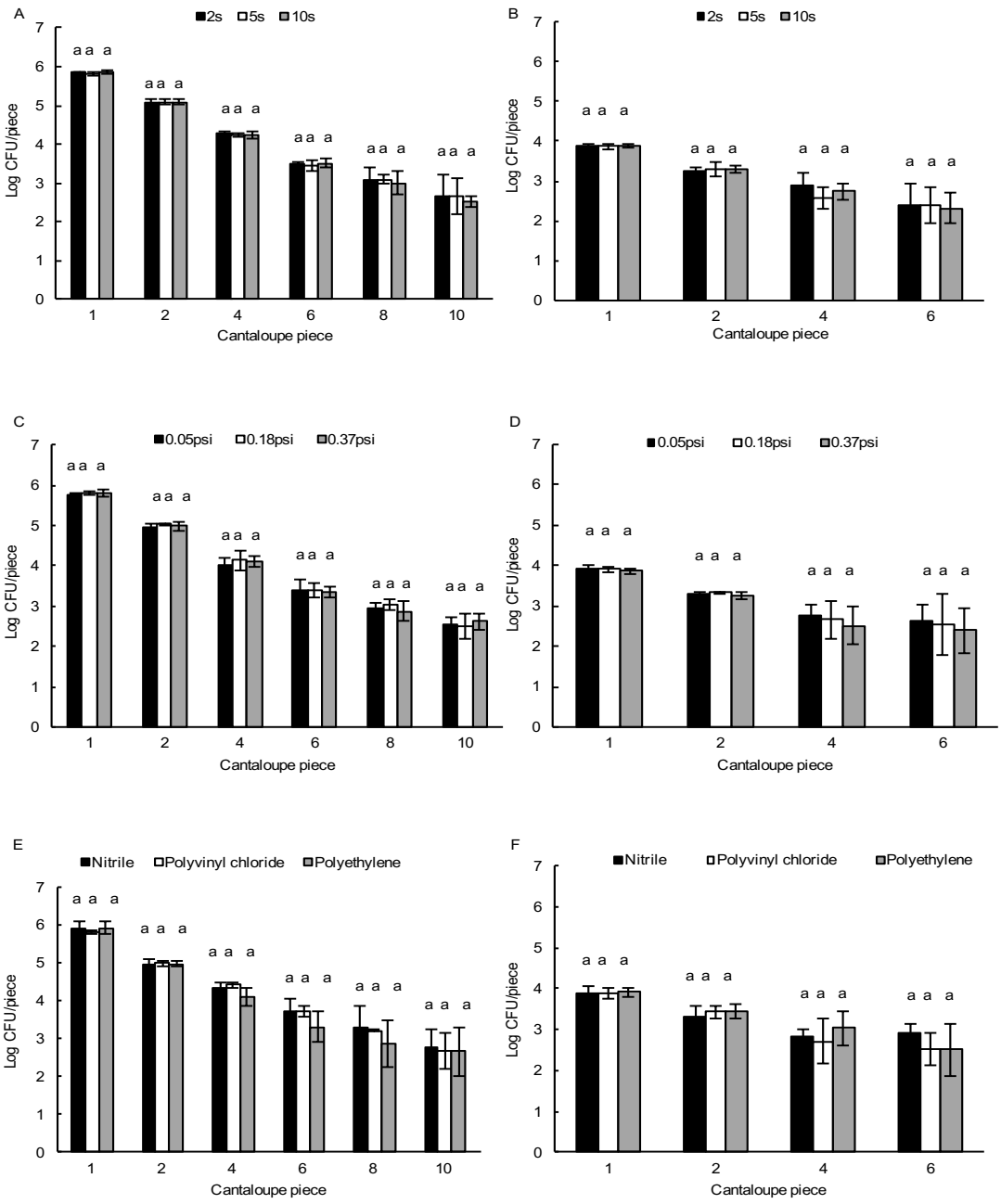

Figure 3.1 Number of *L. monocytogenes* transferred via pressing an inoculated piece of cantaloupe flesh with a gloved hand, followed by consecutively pressing uninoculated pieces. A, C, E and B, D, F represent high inocula (HI) and low inocula (LI), respectively. A-B, C-D, and

E-F show populations of *L. monocytogenes* after transfer from inoculated pieces to consecutive uninoculated pieces as affected by contact time, contact pressure, and glove type, respectively. Within each consecutive piece, the same letter shown indicates no statistical difference ($P > 0.05$) in population of *L. monocytogenes* (n = 6).

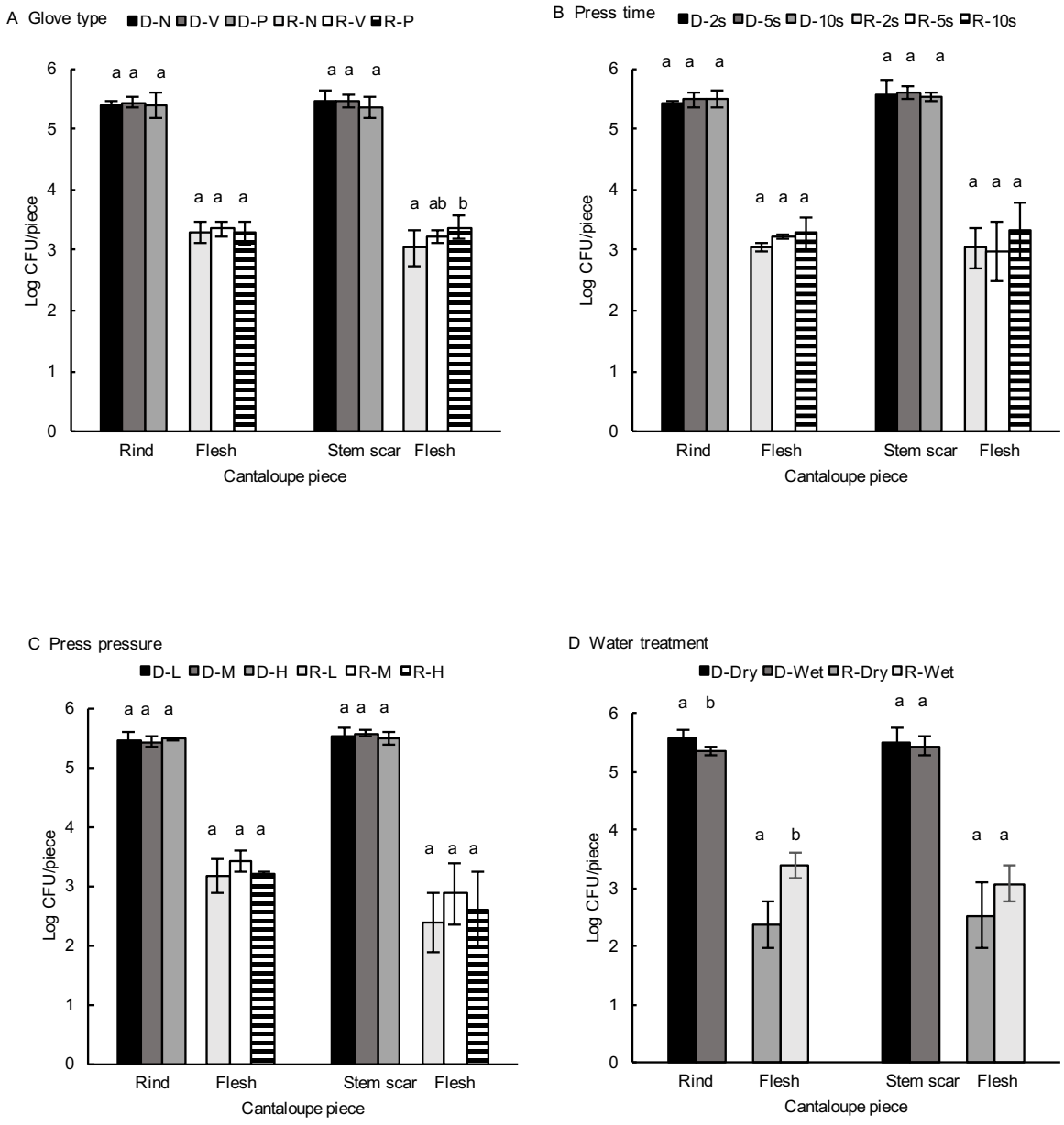

Figure 3.2 Transfer of *L. monocytogenes* from cantaloupe rind or stem scar to flesh via gloved hand. Y-axes show the number (log CFU/piece) of *L. monocytogenes* cells that were left on stem scar or rind pieces [D=donor] and received by flesh pieces [R=recipient] after glove-mediated transfer. Within the same quadrant (A, B, C, or D), type of transfer (rind to flesh or stem scar to flesh), and type of piece, bars with the same letter indicate no statistical difference ($P > 0.05$) in

number of *L. monocytogenes*. Fig 3.2.A shows transfer of *L. monocytogenes* via three types of gloves: nitrile (N), polyvinyl chloride (V), and polyethylene (P) gloves. Fig 3.2.B shows transfer of *L. monocytogenes* as affected by press time: 2 s, 5 s, and 10 s. Fig 3.2.C shows transfer of *L. monocytogenes* via different press pressures: 0.05 psi [L=Low], 0.18 psi [M=Medium], and 0.37 psi [H=High], respectively). Fig 3.2.D shows transfer of *L. monocytogenes* from inoculated cantaloupes rind and stem scar pieces (D) to cantaloupe flesh pieces (R) without [Dry] and with wash [Wet] treatment.

Table 3.1 Regression parameters of three models used to predict transfer of *L. monocytogenes* from cantaloupe flesh containing high (H) or low (L) inocula to uninoculated flesh via three types of gloves

	Glove type											
	Nitrile				Polyvinyl chloride				Polyethylene			
	H		L		H		L		H		L	
Model	R^2	NRMSE	R^2	NRMSE	R^2	NRMSE	R^2	NRMSE	R^2	NRMSE	R^2	NRMSE
$Y = a_1 + b_1 \bullet \ln x$	0.923	0.0825	0.342	0.1812	0.930	0.084	0.535	0.1844	0.862	0.1076	0.606	0.2013
$Y = a_2 \bullet x^{b2}$	0.897	0.0954	0.359	0.1786	0.904	0.0977	0.556	0.1800	0.883	0.0991	0.61	0.2009
$Y = a_3 \bullet e^{(-x/b3)}$	0.880	0.1031	0.181	0.2020	0.881	0.1089	0.385	0.2120	0.726	0.1520	0.529	0.2201

Table 3.2 Equation parameters of three models used to predict transfer of *L. monocytogenes* via three types of gloves from cantaloupe flesh pieces with high inoculum to uninoculated flesh pieces

Parameter	Glove type								
	Nitrile			Polyvinyl chloride			Polyethylene		
	Mean	Lower CI	Upper CI	Mean	Lower CI	Upper CI	Mean	Lower CI	Upper CI
a_1	5.9505	5.7416	6.1595	5.9168	5.6961	6.1376	5.7215	5.4520	5.9910
b_1	-1.2976	-1.3949	-1.2002	-1.3051	-1.4102	-1.2000	-1.2044	-1.3294	-1.0794
a_2	6.1499	5.8702	6.4295	6.1161	5.8173	6.4150	6.0273	5.7388	6.3158
b_2	-0.3248	-0.3526	-0.2970	-0.3281	-0.3588	-0.2973	-0.3234	-0.3526	-0.2941
a_3	5.7260	5.4544	5.9976	5.7365	5.4321	6.0409	5.4006	5.0131	5.7881
b_3	16.5392	14.7990	18.2615	15.7349	13.8712	17.0041	18.2286	15.1617	21.2956

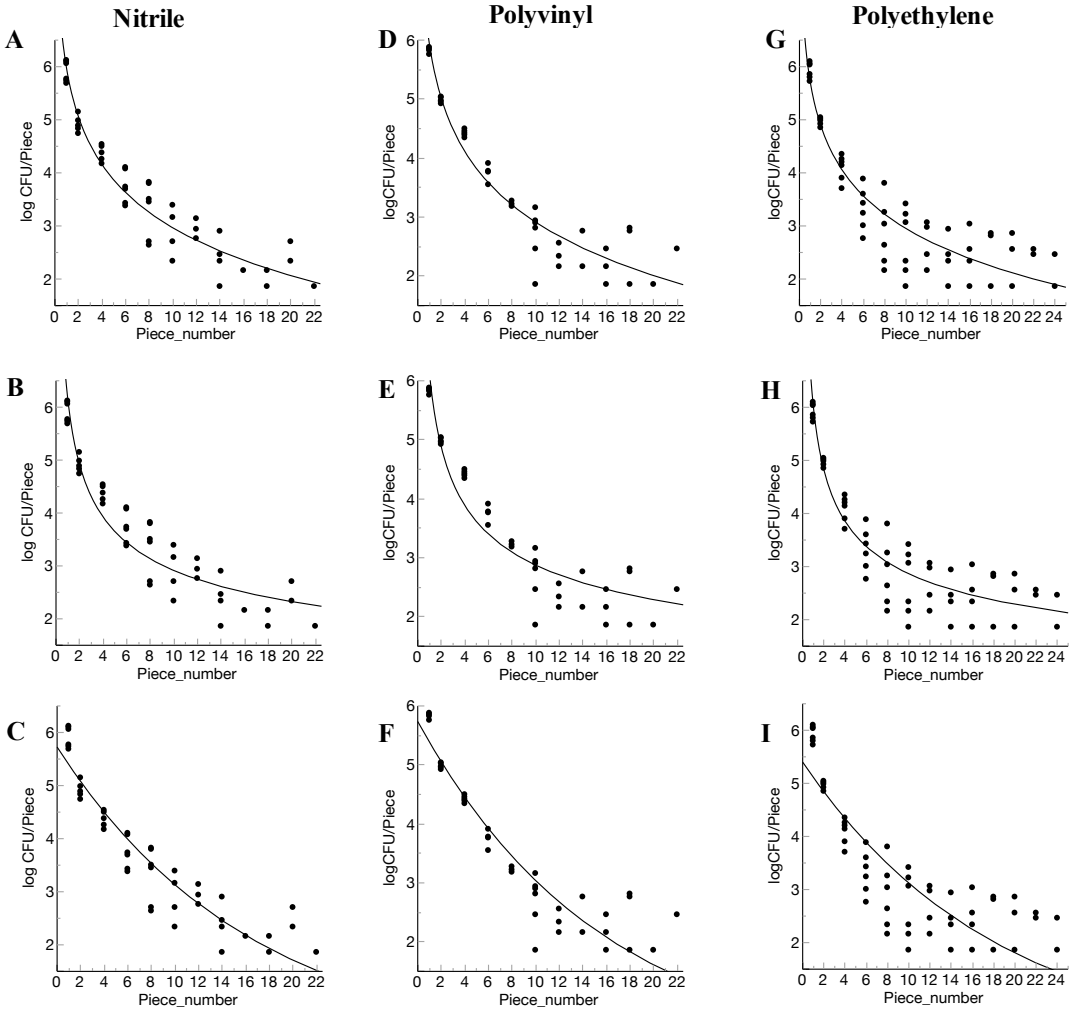

Figure 3.3 Predictive modeling of the number of *L. monocytogenes* in a high inoculum transferred to cantaloupe flesh pieces via sequential pressing with gloved hands. A, B and C show predictive models for transfer via nitrile gloves; D, E and F show predictive models for transfer via polyvinyl chloride gloves; and G, H and I show predictive models for transfer via polyethylene gloves. A, D, and G show the predictive models using Eq[1] $Y = a_1 + b_1 \cdot \ln x$; B, E, and H show predictive models using Eq[2] $Y = a_2 \cdot x^{b2}$; and C, F, and I show predictive models using Eq[3] $Y = a_3 \cdot e^{(-x/b3)}$.

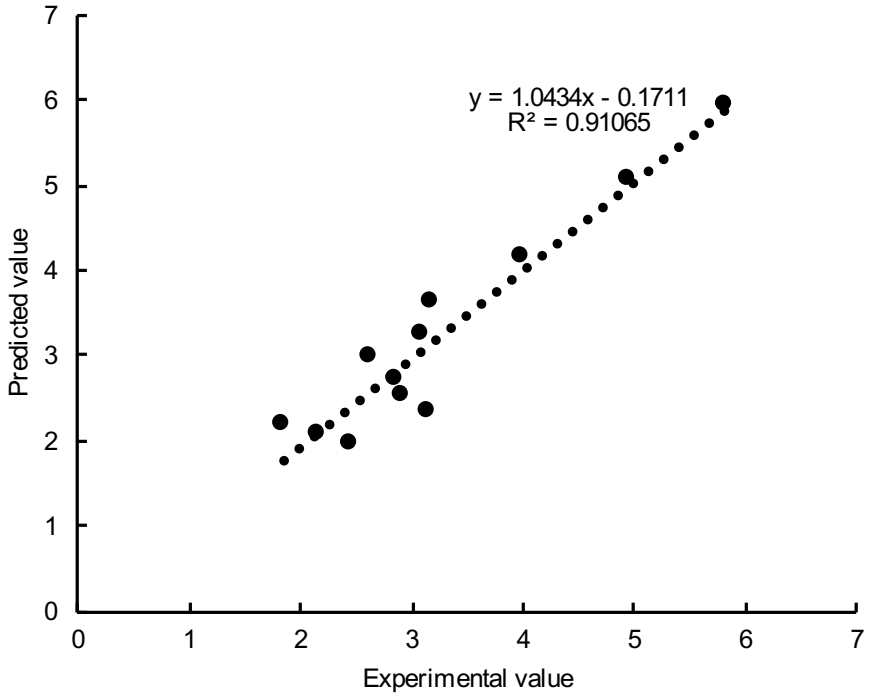

Figure 3.4 Validation plot for the logarithmic model for transfer of *L. monocytogenes* from flesh of cantaloupe pieces initially containing a high inoculum to flesh of uninoculated cantaloupe pieces.

Table.3.3 Comparison of equation parameters of three models used to predict transfer of *L. monocytogenes* from cantaloupe flesh with a low inoculum to uninoculated flesh via gloves

Parameter	Glove type								
	Nitrile			Polyvinyl chloride			Polyethylene		
	Mean	Lower CI	Upper CI	Mean	Lower CI	Upper CI	Mean	Lower CI	Upper CI
a_1	3.6139	3.3644	3.8634	3.7076	3.4477	3.9675	3.8856	3.5911	4.1800
b_1	-0.3295	-0.4523	-0.2067	-0.4898	-0.6229	-0.3566	-0.6412	-0.8083	-0.4742
a_2	3.6616	3.4029	3.9203	3.7815	3.5084	4.0546	3.9518	3.6301	4.2735
b_2	-0.1099	-0.1473	-0.0725	-0.1690	-0.2112	-0.1269	-0.2122	-0.2663	-0.1583
a_3	3.4064	3.1577	3.6550	3.5094	3.2284	3.7905	3.7752	3.4476	4.1028
b_3	67.3794	31.4496	103.3092	36.7514	23.3666	50.1362	22.2705	15.2456	29.2954

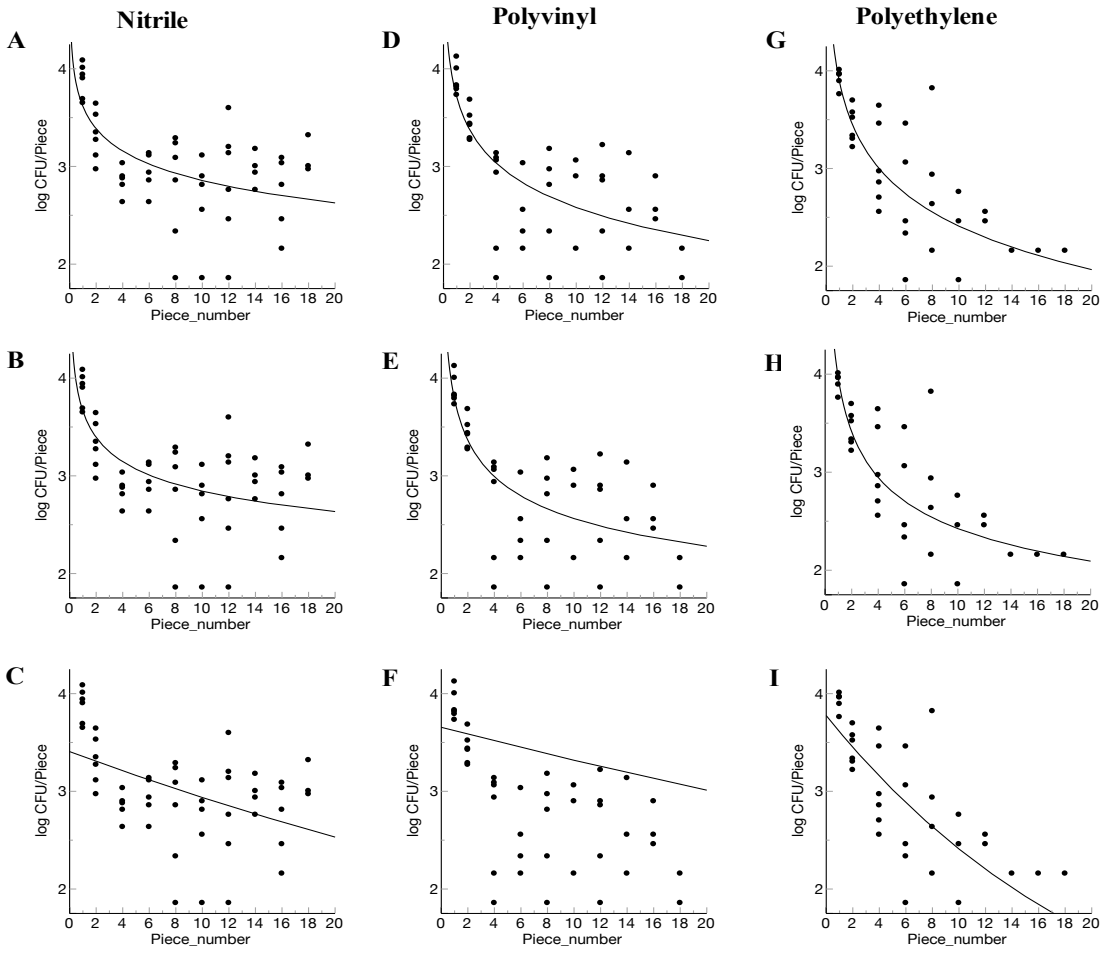

Figure 3.5 Predictive modeling of the number of *L. monocytogenes* in a low inoculum transferred to cantaloupe flesh pieces via pressing with gloved hand. A, B, and C show the predictive model for transfer via nitrile gloves; D, E, and F show the predictive model for transfer via polyvinyl chloride gloves; and G, H, and I show the predictive model for transfer via polyethylene gloves. A, D, and G show the predictive model using Eq[1] ($Y = a_1 + b_1 \cdot \ln x$); B, E, and H show the predictive model using Eq[2] ($Y = a_2 \cdot x^{b2}$) ; and C, F, and I show the predictive model using Eq[3] ($Y = a_3 \cdot e^{(-x/b3)}$).

References

Aarnisalo, K., Sheen, S., Raaska, L., & Tamplin, M. (2007). Modelling transfer of *Listeria monocytogenes* during slicing of 'gravad'salmon. *International Journal of Food Microbiology, 118*, 69-78.

Annous, B. A., Solomon, E. B., Cooke, P. H., & Burke, A. (2005). Biofilm formation by *Salmonella* spp. on cantaloupe melons. *Journal of Food Safety, 25*, 276-287.

Bett-Garber, K., Greene, J., Lamikanra, O., Ingram, D., & Watson, M. (2011). Effect of storage temperature variations on sensory quality of fresh-cut cantaloupe melon. *Journal of Food Quality, 34*, 19-29.

Bialka, K. L., Demirci, A., & Puri, V. M. (2008). Modeling the inactivation of *Escherichia coli* O157: H7 and *Salmonella enterica* on raspberries and strawberries resulting from exposure to ozone or pulsed UV-light. *Journal of Food Engineering, 85*, 444-449.

CDC (2012). Multistate outbreak of listeriosis linked to whole cantaloupes from Jensen Farms, Colorado (final update). *Available at: https://www.cdc.gov/listeria/outbreaks/cantaloupes-jensen-farms/index.html. Accessed on August 14th 2018.*

Chen, D., Zhao, T., & Doyle, M. P. (2014). Transfer of foodborne pathogens during mechanical slicing and their inactivation by levulinic acid-based sanitizer on slicers. *Food Microbiology, 38*, 263-269.

Dickson, J. S. (1990). Transfer of *Listeria monocytogenes* and *Salmonella* Typhimurium between beef tissue surfaces. *Journal of Food Protection, 53*, 51-55.

Doumith, M., Buchrieser, C., Glaser, P., Jacquet, C., Martin, P., (2004). Differentiation of the major *Listeria monocytogenes* serovars by multiplex PCR. *Journal of Clinical Microbiology*, *42*, 3819-3822.

Doumith, M., Jacquet, C., Gerner-Smidt, P., Graves, L. M., Loncarevic, S., Mathisen, T., Morvan, A., Salcedo, C., Torpdahl, M., Vazquez, J. A., & Martin, P.(2005). Multicenter validation of a multiplex PCR assay for differentiating the major *Listeria monocytogenes* serovars 1/2a, 1/2b, 1/2c, and 4b: toward an international standard. *Journal of Food Protection, 68*, 2648-2650.

Erickson, M. C., Liao, J., Cannon, J. L., & Ortega, Y. R. (2015). Contamination of knives and graters by bacterial foodborne pathogens during slicing and grating of produce. *Food Microbiology, 52*, 138-145.

FDA. (2017). Food Code. *Available at: https://www.fda.gov/Food/GuidanceRegulation/RetailFoodProtection/FoodCode/ucm595139.htm Accessed on November 16th 2018.*

Fravalo, P., Laisney, M.J., Gillard, M.O., Salvat, G., & Chemaly, M. (2009). *Campylobacter* transfer from naturally contaminated chicken thighs to cutting boards is inversely related to initial load. *Journal of Food Protection, 72,* 1836-1840.

Garner, D., & Kathariou, S. (2016). Fresh produce–associated listeriosis outbreaks, sources of concern, teachable moments, and insights. *Journal of Food Protection, 79*, 337-344.

Gaul, L. K., Farag, N. H., Shim, T., Kingsley, M. A., Silk, B. J., & Hyytia-Trees, E. (2013). Hospital-acquired listeriosis outbreak caused by contaminated diced celery-Texas, 2010. *Clinical Infectious Disease, 56*, 20-26.

Ivanek, R., Grohn, Y. T., Wiedmann, M., & Wells, M. T. (2004). Mathematical model of *Listeria monocytogenes* cross-contamination in a fish processing plant. *Journal of Food Protection, 67*, 2688-2697.

Jackson, B. R., Salter, M., Tarr, C., Conrad, A., Harvey, E., Steinbock, L., Saupe, A., Sorenson, A., Katz, L., Stroika, S., Jackson, K. A., Carleton, H., Kucerova, Z., Melka, D., Strain, E., Parish, M., & Mody, R.K. (2015). Listeriosis associated with stone fruit-United States, 2014. *Morbidity and Mortality Weekly Report, 64*, 282-283.

Jimenez, M., Siller, J. H., Valdez, J. B., Carrillo, A., & Chaidez, C. (2007). Bidirectional *Salmonella enterica* serovar Typhimurium transfer between bare/glove hands and green bell pepper and its interruption. *International Journal of Environmental Health Research, 17*, 381-388.

Macarisin, D., Wooten, A., De Jesus, A., Hur, M., Bae, S., Patel, J., Evans, P., Brown, E., Hammack, T., & Chen, Y. (2017). Internalization of *Listeria monocytogenes* in cantaloupes during dump tank washing and hydrocooling. *International Journal of Food Microbiology, 257*, 165-175.

Møller, C., Nauta, M., Christensen, B. B., Dalgaard, P., & Hansen, T. B. (2012). Modelling transfer of *Salmonella* Typhimurium DT104 during simulation of grinding of pork. *Journal of Applied Microbiology, 112*, 90-98.

Montville, R., & Schaffner, D. (2003). Inoculum size influences bacterial cross contamination between surfaces. *Applied and Environmental Microbiology, 69*, 7188-7193.

NSW. (2018). Listeriosis alerts. *Available at:* http://www.health.nsw.gov.au/Infectious/diseases/Pages/listeriosis-outbreak.aspx *Accessed on August 14th 2018.*

Parnell, T. L., Suslow, T., & Harris, L. J. (2003). Cantaloupe: safe methods to store, preserve, and enjoy. Publication 8095. Oak-land: University of California, Division of Agriculture and Natural Resources, 1-3.

Patrick, D., Findon, G., & Miller, T. (1997). Residual moisture determines the level of touch-contact-associated bacterial transfer following hand washing. *Epidemiology & Infection, 119*, 319-325.

Pérez-Rodríguez, F., Valero, A., Todd, E., Carrasco, E., García-Gimeno, R., & Zurera, G. (2007). Modeling transfer of *Escherichia coli* O157:H7 and *Staphylococcus* aureus during slicing of a cooked meat product. *Meat Science, 76*, 692-699.

Perkins-Veazie, P., Beaulieu, J. C., & Siddiq, M. (2012). Watermelon, cantaloupe and honeydew. *Tropical and Subtropical Fruits: Postharvest Physiology, Processing and Packaging. John Wiley & Sons, Inc*, 549-568.

Richards, G. M., & Beuchat, L. R. (2004). Attachment of *Salmonella* Poona to cantaloupe rind and stem scar tissues as affected by temperature of fruit and inoculum. *Journal of Food Protection, 67*, 1359-1364.

Ryser, E. T., Marth, E. H., 2007. *Listeria*, Listeriosis and Food Safety, third ed. CRC Press, Boca Raton, FL.

Rico, D., Martin-Diana, A. B., Barat, J., & Barry-Ryan, C. (2007). Extending and measuring the quality of fresh-cut fruit and vegetables: a review. *Trends in Food Science & Technology, 18*, 373-386.

Scollon, A. M., Wang, H., & Ryser, E. T. (2016). Transfer of *Listeria monocytogenes* during mechanical slicing of onions. *Food Control, 65*, 160-167.

Sheen, S. (2008). Modeling surface transfer of *Listeria monocytogenes* on salami during slicing. *Journal of Food Science, 73*.

Shieh, Y. C., Tortorello, M. L., Fleischman, G. J., Li, D., & Schaffner, D. W. (2014). Tracking and modeling norovirus transmission during mechanical slicing of globe tomatoes. *International Journal of Food Microbiology, 180*, 13-18.

Ukuku, D. O., & Fett, W. (2002). Behavior of *Listeria monocytogenes* inoculated on cantaloupe surfaces and efficacy of washing treatments to reduce transfer from rind to fresh-cut pieces. *Journal of Food Protection, 65*, 924-930.

Ukuku, D. O., & Fett, W. F. (2006). Effects of cell surface charge and hydrophobicity on attachment of 16 *Salmonella* serovars to cantaloupe rind and decontamination with sanitizers. *Journal of Food Protection, 69*, 1835-1843.

USDA (2018). National nutrient database for standard reference legacy release. *Available at:* https://ndb.nal.usda.gov/ndb/foods/show/301173?n1=%7BQv%3D1%7D&fgcd=&man=&lfacet=&count=&max=&sort=&qlookup=&offset=&format=Full&new=&measureby=&Qv=1&ds=&qt=&qp=&qa=&qn=&q=&ing= *Accessed on August 14th 2018.*

Webb, C. C., Davey, L. E., Erickson, M. C., & Doyle, M. P. (2013). Evaluation of levulinic acid and sodium dodecyl sulfate as a sanitizer for use in processing Georgia-grown cantaloupes. *Journal of Food Protection, 76*, 1767-1772.

Xiao, X., Pang, H., Wang, W., Fang, W., Fu, Y., & Li, Y. (2018). Modeling transfer of *Vibrio parahaemolyticus* during peeling of raw shrimp. *Journal of Food Science, 83*, 756-762.

Zilelidou, E. A., Tsourou, V., Poimenidou, S., Loukou, A., & Skandamis, P. N. (2015). Modeling transfer of *Escherichia coli* O157: H7 and *Listeria monocytogenes* during preparation of fresh-cut salads: impact of cutting and shredding practices. *Food Microbiology, 45*, 254-265.

CHAPTER 4

TRANSCRIPTOMIC ANALYSIS OF *LISTERIA MONOCYTOGENES* ADAPTATION ON FRESH-CUT PRODUCE[2]

[2] Qi Y and Deng X. To be submitted to *Applied and Environmental Microbiology*

ABSTRACT

In recent years, *Listeria monocytogenes* has been frequently implicated in foodborne outbreaks that linked to fresh and fresh-cut produce. However, knowledge on the molecular mechanisms of *L. monocytogenes* adaptation on fresh-cut produce is still limited. We characterized transcriptomic responses of *L. monocytogenes* on fresh-cut apples, cucumbers, cantaloupes, tomatoes, and celery using transcriptome sequencing or RNA-Seq. Fresh-cut produce samples were inoculated with 10^9 CFU of *L. monocytogenes* strain F8027 that was previously isolated from celery. Samples were stored at 4°C for 48 hours before RNA extraction. A reference sample was prepared by subjecting the same strain in phosphate buffered saline (PBS) under the same storage condition. Total RNA of *L. monocytogenes* was extracted, purified, reverse transcribed to cDNA, and sequenced on an Illumina MiSeq platform. Genes and functional pathways that showed differential expression on fresh-cut produce samples in comparison with the reference or among different produce were identified. RNA-seq results showed that a total of 505 and 440 genes were significantly up- and down-regulated on all the five fresh-cut produce samples (False discovery rate (FDR) < 0.05, fold change≥2 for up-regulated genes, fold change ≤ 0.5 for down-regulated genes). Major functional categories of commonly up-regulated genes included membrane transport, transcriptional regulation, and amino acid metabolism. Major functional categories of commonly down-regulated genes were phosphotransferase system (PTS) and carbohydrate metabolism. Contrast of overall *Listeria* transcriptomes was most pronounced among *L. monocytogenes* cells adapted on fresh-cut cantaloupes, celery, and apples. Differential expression of 656, 229, and 978 genes was uniquely observed on fresh-cut cantaloupes, fresh-cut celery, and fresh-cut apples, respectively. The up-regulated genes and pathways related to cell motility and translation were identified on fresh-cut

cantaloupes. The up-regulated genes and pathways that are associated with sugar metabolism and amino acids biosynthesis were found on fresh-cut celery. The down-regulated genes and pathways including translation and DNA repair were found on fresh-cut apples. The information obtained from this study provided molecular assessment of metabolic and stress responses that are likely important for the pathogen's adaptation and fate on fresh-cut produce, and a better understanding of *L. monocytogenes* adaptation strategies on specific fresh-cut produce. This study provided potential transcriptomic markers and molecular targets for future development of detection and control strategies of *L. monocytogenes* on fresh-cut produce.

Keywords: *L. monocytogenes*, RNA-seq, transcriptome, fresh-cut produce

4.1 Introduction

L. monocytogenes is one of the major foodborne pathogens of serious safety and public health concerns in the United States. It is estimated that 1,600 people contract listeriosis and 260 people die from the infection each year in the US (CDC, 2018). Outbreaks of listeriosis linked to fresh produce have increased in recent years. In 2010, a multistate outbreak of *L. monocytogenes* infections linked to celery caused 10 hospitalizations and 5 deaths (Gaul et al., 2012). In 2011, *L. monocytogenes* contaminated cantaloupe led to one of the deadliest foodborne disease outbreaks in the United States, resulting in 147 illnesses and 33 deaths (CDC, 2012). In 2014, listeriosis infections associated with caramel apples resulted in 35 illnesses and 7 deaths (CDC, 2015).

Multiple reasons may contribute to *L. monocytogenes* contamination of fresh produce. First, *L. monocytogenes* is widely distributed in the natural environment. It can be present in soil, water, decayed vegetation, silage, sewage, and animal feces (Fenlon, 1999; Bhunia, 2018). Fresh produce can be contaminated by these natural sources when growing in fields (Zhu et al., 2017; Bhunia, 2018). For example, fresh-produce can be contaminated with *L. monocytogenes* by sewage water applied as fertilizer to the crops (Zhu et al., 2017; FSAI, 2005). Second, *L. monocytogenes* can survive or grow under a broad range of conditions during food processing or storage. The U.S. Food and Drug Administration (FDA) recommends fresh-cut produce should be refrigerated at 5°C or lower to prevent possible pathogens from multiplying (FDA, 2017). However, this cold environment cannot inhibit *L. monocytogenes* growth. It is reported that *L. monocytogenes* can grow at -0.4°C (Junttila et al., 1988). Third, pH and water activity range commonly found in fresh produce support *L. monocytogenes* growth and survival. For example, Penteado et al. (2004) investigated the growth of *L. monocytogenes* on melon (pH=5.87), watermelon (pH=5.50), and papaya pulps (pH=4.87) at 10°C. They found that *L. monocytogenes*

on cut melons had the generation time of ~7 h and *L. monocytogenes* on papaya showed the generation time of ~15 h (Penteado et al., 2004).

A number of studies have been conducted to examine the fate of *L. monocytogenes* on fresh and fresh-cut produce (Babic et al., 1997; Danyluk et al., 2014; Fang et al., 2013; Penteado & Leitao, 2004; Vandamm et al., 2013; Salazar et al., 2017). Vandamm et al. (2013) investigated the fate of *L. monocytogenes* on fresh-cut celery. They found that populations of this pathogen on fresh-cut celery declined by 0.5-1 log CFU/g at 4°C after incubation for 7 days. Danyluk et al. (2014) modeled and predicted that *L. monocytogenes* on cut melons had a 1 log increase at 4 °C after 6 days incubation. Salazar et al. (2017) assessed the fate of *L. monocytogenes* on common retail cut produce. They reported that cantaloupe flesh and avocado pulp supported the rapid growth of *L. monocytogenes*. In spite of these studies, little is known about molecular mechanisms of *L. monocytogenes* adaptation on fresh-cut produce.

In recent years, transcriptomic analysis of foodborne pathogens on foods has been well documented (Deng et al., 2012; Suo et al., 2018; Tang et al., 2015; Crucello et al., 2019; Goudeau et al., 2012). Transcriptome profiling provides a means of investigating bacterial physiological states in specific food environments and characterizing metabolic activities under environmental stress (Fink et al., 2012). Additionally, the transcriptome of bacteria in food matrices may help to develop preservation technologies to control pathogens in the food system (Liu & Ream, 2008; Mutz et al., 2013, Suo et al., 2018). For example, Tang et al. (2015) investigated physiological states of *L. monocytogenes* on vacuum-packaged cold smoked salmon. One purpose of this study was to identify potential molecular targets for developing better detection methods and control strategies for *L. monocytogenes* on salmon. Deng et al. (2012) studied transcriptomic response of *Salmonella* Enteritidis in peanut oil under desiccation stress.

They found that desiccated and starved *Salmonella* cells entered a seemingly dormant state with less than 5% of *Salmonella* genome still being transcribed, including a few transcripts that might be important for *Salmonella* survival in that environment. Suo et al. (2018) studied transcriptomic response of *L. monocytogenes* on meat treated with sodium lactate. They reported that this treatment inhibited flagella synthesis but increased the pathogenic potential of *L. monocytogenes*. Goudeau et al. (2012) studied the *Salmonella* transcriptome in lettuce and cilantro soft rot. They found that *Salmonella* exposed to a fresh produce environment showed similar gene expressions required to colonize the animal host intestine. This finding revealed that *Salmonella* used similar mechanisms to colonize fresh produce and animal cells.

This study was designed to characterize transcriptomic responses of *L. monocytogenes* on fresh-cut apples, cucumbers, cantaloupes, tomatoes, and celery to probe produce factors and bacterial mechanisms underlying *L. monocytogenes* adaptation on fresh-cut produce.

4.2 Materials and methods

To determine sample type (fresh-cut produce or produce juice) and length of *L. monocytogenes* adaptation for characterizing *L. monocytogenes* transcriptome, we studied the fate of *L. monocytogenes* in fresh-cut produce juice over 7 days and on fresh-cut produce over 3 days at 4°C.

4.2.1 Bacterial strains and preparation of inoculum

To study the fate of *L. monocytogenes* in fresh-produce juice, an inoculum including four strains of *L. monocytogenes* from our culture collection at the Center for Food Safety, University of Georgia was prepared to include: strain F8027 (serovar 4b, a celery isolate), F8369 (serovar 1/2a, a corn isolate), F8255 (serovar 1/2b, a peach isolate), and ATCC 51779 (serovar 1/2c, a cheese isolate). Frozen cultures (-20 °C) were thawed, transferred (10 µl) to 5 ml of tryptic soy

broth (TSB; Becton Dickinson, Sparks, MD) supplemented with 0.6% (wt/vol) yeast extract (Becton Dickinson)(TSBYE) and incubated with shaking (200 rpm) for 18-20 h at 37°C. To reduce background populations in fresh produce juices during *L. monocytogenes* enumeration, cultures were then transferred to 5 ml TSBYE with the presence of 50 μg/ml of nalidixic acid (TSBYEN) incubated with shaking (200 rpm) for 18-20 h at 37 °C. The adapted cultures were streaked on tryptic soy agar (TSA; Becton Dickinson, Sparks, MD) with 0.6% (wt/vol) yeast (Becton Dickinson) (TSAYE) with the presence of 50 μg/ml of nalidixic acid (TSAYEN). A single colony from each plate was transferred to 5 ml of TSBYEN. Cultures were incubated with 200 rpm shaking at 37°C for 16 -18 h. Cultures were then adjusted to an optimal density at 600 nm (OD_{600}) of 0.8 and washed in 1× phosphate buffered saline (PBS; AccuGENE, Rockland, ME). Cell suspensions of each strain were combined to make a mixture of strains containing 8 - 9 log CFU/ml (high inoculum, HI) followed by serially diluting to give a suspension containing 4 - 5 log CFU/ml (low inoculum, LI). These suspensions were used to inoculate fresh-cut produce juice.

A single strain (F8027) was used to study the fate and transcriptome of *L. monocytogenes* on fresh-cut produce. F8027 is a lineage I, serotype 4b strain isolated from celery. Serotype 4b is one of the most frequently implicated serotypes in listeriosis outbreaks (Doumith et al., 2004). *L. monocytogenes* F8027 was transferred from frozen stocks (-80°C) freezer to 5 ml of TSBYE and incubated with shaking (200 rpm) for 18-20 h at 37 °C. The cultures were streaked on TSAYE and incubated at 37°C for 24 h. A single colony was transferred to 15 ml TSBYE and incubated with shaking (200 rpm) for 16 h at 37°C. After 16 h, the cultures were centrifuged at 16,200 × g for 1 min. The cell pellets were washed with 1× PBS twice and resuspended in

1/10 (Vol_{add} /$Vol_{previous}$) PBS. The final *L. monocytogenes* cell concentration was 9 - 10 log CFU/ml.

4.2.2 Preparation of fresh produce samples and sample inoculation

To study the fate of *L. monocytogenes* in fresh produce juices, Gala apples, whole cantaloupes, American cucumbers, spinach, round tomatoes, corns, whole honeydews, and celery were purchased from local markets in Griffin, GA. The fresh produce was thoroughly washed with tap water, dried, cut, and ground with a juicer. Produce homogenates were centrifuged twice for 15 min at 3,000× g to remove sizeable particulate debris. Then the fresh produce juice was filtrated through a circular filter membrane (47 mm diameter, 0.45 μm pore size) (GE Healthcare Life Sciences, Piscataway, NJ).

A 250 μl aliquot of each juice sample was put into each well of a 96-well plate. Uninoculated produce juice samples were included to determine the levels of native microflora. The produce juice sample in each well was inoculated with 2.5 μl of the four-strain mixture. Mixtures of strains containing 8-9 log CFU/ml and 4-5 log CFU/ml were used separately in this experiment.

To study the fate of *L. monocytogenes* on the surfaces of fresh-cut produce, Gala apples, whole cantaloupes, red round tomatoes, American cucumbers, and celery were purchased from local retail supermarkets in Griffin, GA. The fresh produce was stored in walk-in-cooler (4°C) one day before inoculation. All the fresh produce was washed thoroughly with tap water to remove external organic material before cutting. Sterile stainless steel knives were used to cut apples, cantaloupes, tomatoes, and cucumbers into circular pieces and cut celery into rectangular shapes for future use.

Ten drops (100 μl in total) of 9-10 log CFU/ml of *L. monocytogenes* F8027 were evenly spread over a piece of circular nitrile cellulose filter membrane (47 mm diameter, 0.45 μm pore size) (GE Healthcare Life Sciences, Piscataway, NJ). The inoculated filter membrane was sandwiched between two pieces of the same fresh-cut produce. For PBS sample, one hundred microliters of *L. monocytogenes* strain was inoculated in 900 μl of 1×PBS.

Produce juice pH value was determined. A pH meter was calibrated with 4.0 and 7.0 standards. Then glass electrode was inserted into a 25 ml of uninoculated fresh produce juice. Three replicates were conducted.

4.2.3 Microbial and statistical analysis

In fresh produce juice study, samples in 96-well plates were incubated at 4°C for 1, 3, 5, 7 d. At each sampling point, a 100 μl of juice sample in each well was obtained, and serial dilutions were made with 1×phosphate buffered saline (PBS) buffer. The samples were plated onto TSAYEN. Control samples were plated onto TSAYE and TSAYEN to enumerate background microflora. The plates were incubated at 37°C for 48 h. Populations of *L. monocytogenes* determined by plating were expressed as CFU/ml. All experiments were conducted with two replicates samples in triplicate experiments (n = 6). Average numbers and standard deviations were calculated using Excel Microsoft, Redmond, WA).

In the fresh-cut produce study, inoculated samples were kept at 4°C for 0, 8, 24, 48, and 72 h. At each time point, triplicate samples for each produce type were separately processed to harvest *L. monocytogenes* cells. Specifically, each filter membrane between two pieces of fresh-cut produce was retrieved and placed in a sterile 50 ml conical tube using a sterile tweezer. One milliliter of PBS was added to the tube and the tube was vortexed for 1 min to wash the cells off the filter membrane. The rinsates were appropriately diluted, plated onto TSAYE agar, and

incubated at 37°C for 48 h prior to enumeration. Populations of *L. monocytogenes* determined by plating were expressed as CFU/sample. All experiments were conducted in triplicate experiments. Average numbers and standard deviations were calculated using Excel Microsoft, Redmond, WA).

4.2.4 *L. monocytogenes* RNA isolation

Total RNA was purified from *L. monocytogenes* cells harvested from the 48 h fresh-cut produce and PBS samples. To collect enough cells to isolate total RNA, we combined six pieces of filter membranes from fresh-cut produce samples or six PBS samples. An aliquot of 1,000μl PBS dilution of RNA protection reagent (Qiagen, Valencia, CA) (66.6% (v/v) RNA protection reagent, 33.3% PBS (v/v)) was immediately added to the harvested cells. The mixture was vortexed and incubated at room temperature for 10 min. The cell suspension was then centrifuged at 16,200 ×g for 1 min to pellet cells. Total RNA from each sample was isolated using Ambion Ribopure bacterial kit (Life Technologies, Carlsbad, CA). RNA quantity was assessed using the Qubit RNA broad range kit as well as the Qubit 2.0 fluorometer (Invitrogen, Grand Island, NY). RNA integrity was analyzed using a 2100 bioanalyzer (Agilent, Foster City, CA). Total RNA samples with an RNA integrity number (RIN) over 8 were kept. All experiments were performed in duplicate.

4.2.5 cDNA preparation and RNA-seq

16S rRNA and 23S rRNA were removed from total RNA to enrich mRNA. 1-5μg total RNA was treated with Ribo-zero rRNA Removal Kit (Bacteria) (Illumina, San Diego, CA). Treated total RNA samples were assessed with an Agilent 2100 bioanalyzer to confirm if 16S rRNA and 23 S rRNA had been removed. The resulting mRNA samples were reverse transcribed to cDNA samples using a Truseq Stranded mRNA Sample Preparation Kit (Illumina, San Diego,

CA). cDNA libraries were sequenced on an Illumina Miseq platform using Miseq Reagent Kit v3 (paired end, 300 bp per read) (Illumina, San Diego, CA).

4.2.6 RNA-seq analysis

The overall quality of sequencing reads was assessed by fastqc. Sequencing reads were trimmed to remove the low quality reads and adaptor sequences using trimmomatic tool (Bolger et al., 2014). Since the F8027 genome has not been fully assembled, sequencing reads were aligned to the closed genome of *L. monocytogenes* F2365 using TopHat2 (version 2.1.1) (Kim et al., 2013) with default settings. Cufflinks quantified the expression of each gene using default parameters and reported it as fragments per kilo bases per million reads (FPKM) (Trapnell et al., 2012). Principal component analysis (PCA) was conducted using FPKM to check the reproducibility and overall expression of each pair of samples.

To conduct differential gene expression analysis, the number of reads mapped to each F2365 gene was counted with Htseq-count (Anders et al., 2015). After Htseq-count analysis, *L. monocytogenes* genes that showed differential expression on fresh-cut produce samples in comparison with the PBS-treated reference or fresh-cut produce sample reference were identified using edgeR package (Robinson et al., 2010). Genes were considered to be significantly regulated if they had a false discovery rate < 0.05 and a fold change of expression (FC) ≥ 2 for genes up-regulated or a FC ≤ 0.5 for genes down-regulated on fresh produce samples.

4.2.7 Gene set enrichment analysis

Gene Set Enrichment Analysis (GSEA) was used to identify pre-defined gene sets exhibiting statistically significant up- or down-regulation between samples (Subramanian et al., 2005). Gene sets were defined according the Kyoto Encyclopaedia of Genes and Genome (KEGG) database (Kanehisa et al., 2000) and obtained from using the gage package of R (Luo et

al., 2009). These gene sets include metabolic pathways, cellular processes, genetic information processing, and environmental information processing. Gene expression fold changes of produce samples compared with PBS reference samples (log_2FC) were used for GSEA analysis. An entire KEGG pathways was considered to be differentially regulated if it met the criteria of false discovery rate (FDR) < 0.25 and a $P < 0.05$.

4.3 Results

4.3.1 Fate of *L. monocytogenes* on fresh-cut produce surfaces and in fresh-cut produce juices

The pH values of the fresh-cut produce (juices) used in this study were shown in Table 4.1. Changes in population of *L. monocytogenes* from 0-day to 7-day displayed a direct correlation with the pH of fresh produce juice ($R^2 = 0.83$ for 5 log CFU/ml and $R^2 = 0.71$ for 9 log CFU/ml) (Figure 4.1-2).

At the two inoculum levels (5 log CFU/ml and 9 log CFU/ml), *L. monocytogenes* was able to grow in honeydew, celery, spinach, corn, and cantaloupe juice at 4°C for 7 days. Populations of *L. monocytogenes* remained constant in cucumber and tomato juice. However, *L. monocytogenes* cannot survive in apple juice at 4°C for more than 3 days (Figure 4.3-4).

Populations of *L. monocytogenes* remained stable on fresh-cut produce and in PBS for 3 days (Figure 4.5). At 3 days, *L. monocytogenes* was able to grow in cantaloupe juice and celery juice but remained constant on fresh-cut cantaloupe and celery. *L. monocytogenes* cell populations remained constant in cucumber and tomato juice as well as on fresh-cut cucumbers and tomatoes (Figure 4.3-5). *L. monocytogenes* did not survive in apple juice for more than 3 days but was able to persist on fresh-cut apple at 4°C for 3 days (Figure 4.3-5).

Harvested *L. monocytogenes* cell populations on all fresh-cut produce were significantly lower than that in PBS ($P<0.05$). It is possible that some of the *Listeria* cells were attached to the fresh-cut produce or filter membrane and not transferred to the rinsate. According to the survey in local supermarkets, the shelf life for fresh-cut produce, especially fresh-cut melon, is ~3 days. To characterize *L. monocytogenes* transcriptome on fresh-cut produce during its retail shelf life, we sampled *Listeria* RNA after 48 h of *in situ* adaptation on fresh-cut produce. When RNA was isolated, the average *L. monocytogenes* population was 10.17±0.04 log CFU/sample in PBS buffer, 9.83±0.10 log CFU/sample on cucumber, 9.46±0.26 log CFU/sample on tomato, 9.21±0.25 log CFU/sample on cantaloupe, 9.48±0.23 log CFU/sample on apple, and 9.51±0.04 log CFU/sample on celery (Figure 4.5).

4.3.2 Overall transcriptomes of *L. monocytogenes* on fresh-cut produce and in PBS

Principal component analysis was performed on the overall *Listeria* transcriptomes on fresh-cut produce and in PBS. Replicate transcriptomes on the same produce clustered (Figure 4.6), suggesting good reproducibility in characterizing overall transcriptomes as *L. monocytogenes* cells adapted on different fresh-cut produce.

L. monocytogenes transcriptome in PBS showed a higher percentage of tRNAs (68.8%) and a lower percentage of protein-coding genes (4.3%) than those on all fresh-cut produce samples. Major functional categories of most abundant protein-coding transcripts (top 1% of protein-coding genes) in PBS included hypothetical proteins (n=10), domain-containing protein (n=4), stress response genes (n=4), and transcriptional regulation (n=2) (Table 4.2). Among tested fresh-cut produce samples, the highest abundance of tRNAs (43.5%) and the lowest abundance of protein-coding genes (13.8%) were both observed on apples. In sharp contrast to apples, cantaloupes induced the highest percentage of protein-coding genes (55.0%) and the

lowest percentage of tRNAs (11.0%) (Figure 4.7). A total of 9 protein-coding genes were among of the top 1% most transcribed genes on all fresh-cut produce samples. Major functional categories of these genes include hypothetical proteins (n=5), stress response proteins (n=3), and ribosomal protein (n=1) (Table 4.3).

4.3.3 Comparison between *L. monocytogenes* transcriptomes on fresh-cut produce and in PBS

Compared to the reference sample in PBS, a total of 505 and 440 genes were significantly up- and down-regulated on all five fresh-cut produce, respectively (a fold change of expression (FC) ≥2 for up-regulated genes or a FC ≤ 0.5 for down-regulated genes, $P < 0.05$, FDR<0.05) (Figure 4.8). Major functional categories of commonly up-regulated genes included amino acids biosynthesis (n=40), ABC transporters (n=22), and transcriptional regulation (n=42) (Table S1). Major functional categories of commonly down-regulated genes were carbon metabolism (n=22), starch and sucrose metabolism (n=26), phosphotransferase system (n=29), and virulence (i.e., *plcA*, *hly*, *actA*, and *plcB*) (Table S2).

The upregulated genes led to the identification of seven metabolic pathways that were significantly upregulated on all fresh-cut produce samples ($P < 0.05$, FDR <0.25) . Multiple of these upregulated pathways mediate metabolism and biosynthesis of amino acids, including cysteine, methionine, and lysine. (Table 4.4, Figure 4.9). Based on commonly downregulated genes, eight metabolic pathways were identified to be significantly downregulated on all fresh-cut produce samples (P <0.05, FDR <0.25). All these pathways were related to carbohydrate metabolism, including pentose phosphate pathway, carbon metabolism, and phosphotransferase system (Table 4.4, Figure 4.10).

The expression of 24 genes was not detected in PBS but on all fresh-cut produce samples (Gene transcript levels (FPKM) >20). These genes encode proteins such as hypothetical proteins (n=6), domain-containing protein (n=3), and transcriptional regulators (i.e., Crp/Fnr family transcriptional regulators, transcriptional regulators, and MarR family transcriptional regulator) (Table 4.5).

Among all tested produce samples, fresh-cut apple stood out for the largest numbers of differentially regulated genes that were sample specific, including 132 up-regulated and 208 down-regulated genes that were unique in apple (Figure 4.8). Major functional categories of uniquely up-regulated genes were transcriptional regulation (n=14), reduction and oxidation reaction (n=5), quorum sensing (n=3), and two components system (n=3) (Table S3). Major functional categories of down-regulated genes encoded proteins involved in ribosome (n=17), purine metabolism (n=10), and aminoacyl-tRNA biosynthesis (n=8) (Table S4).

After apples, cantaloupes had the second highest numbers of up-regulated (n= 35) and down-regulated (n=22) genes. One example of uniquely up-regulated genes on cantaloupes was cell motility bioprocess such as those involved in flagellar assembly (Table 4.6).

4.3.4 Pairwise comparison of *L. monocytogenes* transcriptomes on fresh-cut produce

According to principal component analysis of overall transcriptomes, the largest transcriptomic divergences were observed among apple, cantaloupe, and celery samples. Pairwise transcriptome comparison was conducted among the three samples to identify produce-specific transcriptomic responses (Figure 4.11).

A total of 388 and 268 genes were identified as up- and down-regulated, respectively, on fresh-cut cantaloupes compared with fresh-cut celery and apples (Table S5-S6). Ten pathways were significantly up-regulated on cantaloupes. These up-regulated genes and pathways were

related to cell motility (e.g., flagellar assembly), virulence (*hly*, *plcA*, *plcB*, *actA*), translation (e.g., ribosome), DNA repair (e.g., mismatch repair) as well as energy (e.g., oxidative phosphorylation), vitamins (e.g., One carbon pool by folate), nucleotide (e.g., purine), and glycan (e.g., peptidoglycan) metabolism (Table 4.7, S5).

A total of 158 and 71 genes were up- and down-regulated, respectively, on fresh-cut celery as compared to fresh-cut apples and cantaloupes (Table S7-S8). Five up-regulated pathways were identified during *L. monocytogenes* adaptation on fresh-cut celery (Table 4.7). The up-regulated genes and pathways were associated with carbohydrate transport and utilization (e.g., phosphotransferase system, starch and sucrose metabolism), vitamins metabolism (e.g., pantothenate and CoA biosynthesis), and amino acid biosynthesis (e.g., valine, leucine and isoleucine biosynthesis) (Table 4.7, Table S7).

A total of 978 genes showed differential expression (fold change≥2 for up-regulated genes, fold change ≤ 0.5 for down-regulated genes, $P < 0.05$, FDR < 0.05) during *L. monocytogenes* adaptation on fresh-cut apples versus fresh-cut cantaloupes and celery, including 453 genes that were upregulated and 525 genes that were downregulated (Table S9-S10). Additionally, seven pathways were down-regulated on apple. The downregulated genes and pathways were associated with translation (e.g., ribosome), nucleotide metabolism (e.g., pyrimidine metabolism), DNA repair (e.g., mismatch repair), glycan metabolism (e.g., peptidoglycan metabolism), carbohydrate metabolism (e.g., C5-branched dibasic acid metabolism), drug resistance (e.g., Cationic antimicrobial peptides resistance), cell wall surface anchor, and PrfA regulon (*hly, plcA, actA*) (Table 4.7, S9-S10).

4.3.5 Nutritional properties of investigated fresh-cut produce

Nutritional properties of investigated fresh-cut produce were compared (Table 4.8, Figure 4.12). Comparison of nutrient levels including carbohydrates, amino acids, and fatty acids was based on USDA National Nutrient Database for Standard Reference (USDA, 2018a, 2018b, 2018c, 2018d, 2018e). Comparison of total phenolic levels was based on previous studies (Athunibat et al., 2009; Dewanto et al., 2002; Isabelle et al., 2010; Yao & Ren, 2011). Apples have the highest levels of carbohydrates (13.81g/100g), glucose (2.43g/100g) and total phenolic (1.25 mg/GAE/g) but the lowest level of amino acids (0.201g/100g). Cantaloupe ranks the highest in methionine (0.012g/100g) and branched chain amino acids branched chain amino acids (valine, isoleucine, leucine) (0.083 g/100g) but the lowest in total phenolic (0.11mg/GAE/g). Celery has the highest level of fatty acids (0.153g/100g) but the lowest levels of carbohydrates (2.97g/100g) and glucose (0.4g/100g). Total amino acids are most abundant in tomatoes (0.865g/100g). Fatty acids are least abundant in cucumbers (0.074g/100g).

4.4 Discussion

4.4.1 The choice of produce and reference samples to characterize and compare *L. monocytogenes* transcriptomes

The fate of *L. monocytogenes* in produce juice differs from that on fresh-cut produce. Conway et al. (2000) studied growth and survival of *L. monocytogenes* on fresh-cut apples and apple juice. They found that populations of *L. monocytogenes* declined over time in apple juice but remained stable on fresh-cut apple slices at 5°C. Consistent with their findings, we found that *L. monocytogenes* could not survive in apple juice for three days but persisted on fresh-cut apples for three days. It was suggested by Conway et al. (2000) that *L. monocytogenes* could modify and adapt to the immediate micro-environment on apple slices. However, *L. monocytogenes* cannot modify the liquid environment such as apple juice (Conway et al., 2000).

In previous investigations of bacterial transcriptome in food matrices, liquid food samples or their surrogates were often used to facilitate the separation and harvest of bacterial cells from food matrices. For example, Deng et al. (2012) used peanut butter oil instead of peanut butter to study *Salmonella* Enteriditis in low moisture food under desiccation and starvation stress. In the current study, because of the different fates of *L. monocytogenes* on fresh-cut apples versus in apple juice, it would be inappropriate to use apple juice as a surrogate to study *L. monocytogenes* transcriptome on fresh-cut apples.

In previous transcriptomic studies, bacterial transcriptomes in growth media were commonly used as the reference with which food-induced transcriptomes were compared. The nutritious conditions provided by these media are rarely found in food production, processing and storage environments. The reference transcriptomes under these conditions may hardly reflect the physiological states of foodborne pathogens at any stage along the farm-to-fork spectrum, where various nutrients are often limited or absent. Therefore, in this study, we used *L. monocytogenes* transcriptome in PBS buffer as the reference. Devoid of nutrients, PBS provides a more realistic reference environment to simulate nutrient starvation for *L. monocytogenes*. It was also expected that a nutrient-deprived reference would allow more sensitive detection of transcriptomic responses to nutrients in fresh-cut produce, especially when low level of nutrients elicit subtle but important changes in gene expression.

4.4.2 *L. monocytogenes* transcriptome in PBS

L. monocytogenes cells appeared to be metabolically active in PBS with 89.50% of all protein-coding genes being transcribed. PBS solution is a nutrient deprived environment and essential nutrients (e.g., glucose, amino acids, and vitamins) are not available. However, *L. monocytogenes* was able to survive in PBS for at least three days. One possible explanation is

that *L. monocytogenes* cells can recycle and utilize intracellular nutrients released from the cells. First, the lysis of dead cells may release nutrients for the remaining cells to consume. Lungu et al. (2010) studied *L. monocytogenes* survival in PBS and reported an increase in nucleic acids and proteins in PBS for the first eight days. They suggested that the dead cells can serve as a source of proteins and nucleic acids (Lungu et al. 2010). Second, live bacterial cells may also release nutrients under certain conditions. Shimizu (2014) proposed that when *Escherichia coli* cells are exposed to a nutrient deprived environment, intracellular nutrients may diffuse out due to the different osmotic pressures between cytosol and extracellular environment. Then *E. coli* cells rely on active transport systems to pump in scarce nutrients such as phosphate and ammonium from the extracellular environment for survival. However, whether nutrient diffusion driven diffusion of nutrients exist in gram-positive organisms such as *L. monocytogenes* remains to be investigated.

In addition, the upregulation of pentose phosphate pathway may facilitate the anabolism in *L. monocytogenes* cells which appeared to be metabolically active. We found that up-regulated genes encoded enzymes that are associated with the nonoxidative branch of pentose phosphate pathway (PPP) (Figure 4.11). This branch can synthesize ribose as well as other sugars. Also, Slaghuis et al. (2007) reported that the production of erythrose-4-phosphate from the nonoxidative chain is used for biosynthesis of aromatic amino acids that are not in the minimal medium.

Amino acids deprivation in PBS may result in overexpression of tRNA transcripts, which may contribute to transcription of *L. monocytogenes* in PBS. *Listeria* transcriptome in PBS had a much larger percentage of tRNA transcripts (68.8%) including both charged tRNAs (aminoacyl-tRNAs) and uncharged tRNAs than the transcriptomes on fresh-cut produce. Raina

and Ibba (2014) reported that during amino acid starvation, uncharged tRNAs in Gram-positive bacteria can stabilize the structure of anti-terminators and prevent the competing terminator formation, and then the full-length of mRNA can be transcribed. Aminoacyl-tRNA molecules are required for protein synthesis. As lower transcript levels of protein-coding genes were found in PBS, we propose that charged tRNAs (aminoacyl-tRNAs) accounted for the small portion and uncharged tRNAs were in the large portion of tRNAs in PBS. The larger percentage of uncharged tRNAs was possibly used for promoting *L. monocytogenes* transcription in PBS.

4.4.3 *L. monocytogenes* adaptation on fresh-cut produce

4.4.3.1 Common transcriptomic responses during L. monocytogenes adaptation on fresh-cut produce.

L. monocytogenes regulated amino acid metabolism and transportation as well as sugar transportation during its adaptation on all tested fresh-cut produce samples in comparison with its survival in PBS. In addition, *L. monocytogenes* may develop the ability of attachment to tested fresh-cut produce environment.

We observed that lysine and methionine biosynthesis pathways in *L. monocytogenes* were upregulated on all fresh-cut produce (Table 4.7, Figure 4.10). Lysine biosynthesis can contribute to *L. monocytogenes* cell wall formation (Dogovski et al., 2009). Methionine biosynthesis in *L. monocytogenes* may help to provide the constituent that is used for DNA formation or cellular energy transfer (Chiang et al., 1996; Ferla & Patrick, 2014). These biosynthesis pathways can use glutamine that is commonly found in fresh-cut produce (Ackermann, et al., 1992; Lamikanra, et al., 2000; Pratta, et al., 2004; Roosta, et al., 2009; Schneider, et al., 2003). We also found the gene encoding glutamine ABC transporter permease that is responsible for glutamine transportation was up-regulated by 3.8-14.47 folds on tested fresh-cut produce. The degradation

product of glutamine is glutamate. Glutamate can be converted into L-aspartate which can be used for synthesizing methionine and lysine (Kanehisa et al., 2000). In addition, we also observed that genes and pathways responsible for histidine biosynthesis were up-regulated. The process of histidine biosynthesis provides initial substrates (PRPP and ATP) which can be used for nucleobases, amino acid synthesis, and vitamin synthesis (Alifano et al., 1996; Kang et al., 2019).

Our results suggest that on fresh-cut produce *L. monocytogenes* prioritized the utilization of glucose over other carbohydrates commonly found in produce, such as fructose, sucrose, and maltose (USDA, 2018a, 2018b, 2018c, 2018d, 2018e). On all fresh-cut produce tested, *L. monocytogenes* downregulated starch and sucrose metabolism as well as phosphotransferase systems (PTS), which mediate the uptake of various carbohydrates. However, the gene encoding PTS glucose permease that is responsible for glucose transportation (i.e., glcU) was upregulated. Also, we found that genes encoding PTS mannose permease (i.e., LMOF2365_RS03990, LMOF2365_RS03995) were up-regulated. Stoll et al. (2010) reported that these two genes were also able to transport glucose. These phenomena indicate that *L. monocytogenes* has a preference to utilize glucose over other sugars in a glucose rich environment. Consistent with our results, Gilbreth et al. (2004) reported that the metabolism of mannose, cellobiose, and maltose in *L. monocytogenes* 10403 S were repressed in the presence of glucose. These sugars could only be fermented after glucose was consumed (Gilbreth et al., 2004).

The gene encoding Crp/Fnr family regulator was highly expressed, suggesting that *L. monocytogenes* may increase surface attachment to adapt to fresh-cut produce. The gene (LMOF2365_RS03835) that encodes one of the cyclic AMP receptor protein-fumarate and nitrate reduction family transcriptional regulators (Crp/Fnr) was only expressed on tested fresh-

cut produce but not in PBS. Salazar et al. (2013) investigated the role of Lmo0753 belonging to Crp/Fnr family regulators on *L. monocytogenes* environmental persistence and reported that Lmo0753 is important for *L. monocytogenes* attachment to fresh produce (Salazar et al. 2013). In our study, the protein encoded by gene (LMOF2365_RS03835) is in the same functional group as Lmo0753. Also, there is the amino acid sequence similarity between Lmo 0753 and the protein encoded by gene (LMOF2365_RS03835). Hence, we hypothesize that the expression of the gene LMOF2365_RS03835 may promote *L. monocytogenes* attachment to the tested fresh-cut produce. However, further gene functional studies in terms of bacterial attachment ability still need to be investigated.

In contrast to the finding by Kang et al. (2019) that virulence potential of *L. monocytogenes* was increased as a consequence of growth in cantaloupe, we found lower transcript levels of virulence genes (i.e., *plcA*, *plcB*, *actA*, *hly*) in *L. monocytogenes* on fresh-cut produce. The observed differences are possibly due to the different choices of reference samples. There are substances in produce that may inhibit virulence genes. First, iron that is present in all the tested produce (USDA, 2018a, 2018b, 2018c, 2018d, 2018e) can repress the expression of *Listeria* virulence factors. Böckmann et al. (1996) reported that the presence of iron may strongly inhibit *hly* and *actA* promoter functions. Second, sugar contents in fresh-cut produce affect expression of virulence genes. Gilbreth et al. (2004) showed that in *L. monocytogenes* strain 10403S, sugars such as glucose or cellobiose can repress expression of *hly*. Datta et al. (1993) observed that expression of *hly* was reduced with a higher concentration of glucose in the growth media. Also Ripio et al. (1997) showed that the utilization of glucose and cellobiose can significantly (> 200-fold) inhibit expression of *plcB*. In sum, according to previous studies, we

propose that virulence potential may not be increased during *L. monocytogenes* growth on fresh-cut produce.

4.4.3.2 Produce-specific transcriptomic responses

Produce properties, such as different levels of glucose, pH, amino acids, and antimicrobials, may affect *L. monocytogenes* metabolic activities. We propose that *L. monocytogenes* adopts different strategies to adapt to specific fresh produce environments.

Apple

The abundance of glucose in apples may repress the expression of the gene encoding *Listeria* adhesion protein (LAP). *Listeria* adhesion gene contributes to adherence and intracellular replication (Reddy et al., 2016).We identified that genes encoding cell wall surface anchor proteins showed upregulations on celery or cantaloupe compared with those in *L. monocytogenes* on apple. One of these genes (LMOF2365_RS10635) encodes *Listeria* adhesion protein (LAP). Consistent with our results, Jaradat et al. (2002) showed that nutrition-rich media and high concentrations of glucose can suppress LAP expression.

Lower levels of amino acids possibly lead to a larger percentage of tRNA transcripts which may promote *L. monocytogenes* transcription in apple. Compared with other fresh-cut produce, apple has the lowest concentration of amino acids (0.201/100g) (USDA, 2018a, 2018b, 2018c, 2018d, 2018e). In our study, we observed that tRNAs accounted for the highest (43.5%) percentage of transcriptome of *L. monocytogenes* in apple. This high percentage of tRNA may possibly be used to promote transcription of *L. monocytogenes*. This is supported by Raina and Ibba (2014) that during amino acid starvation, tRNAs can help to keep the terminator functioning well and then the full-length of mRNA can be transcribed.

L. monocytogenes may upregulate genes involved in redox reactions in response to phenolic substances in apple. Our data showed that genes encoding Spx transcriptional regulator (FC=4.54), NADH oxidase (FC=2.99), and superoxide dismutase (FC=2.04) were uniquely up-regulated on apple. Spx global regulator can activate and repress the transcription of a set of genes to respond to oxidative stress (Whiteley et al., 2017; Zuber, 2004). This transcriptional regulator can positively regulate genes encoding superoxide dismutase and NADH oxidase. These genes are involved in defense against oxidative stress (Kajfasz et al., 2010). In our study, the oxidative stress is possibly caused by the oxidant present in apple. One of the oxidants in apple is O-Quinone, which is produced by an abundance of phenolic compounds as well as polyphenol oxidase in fresh-cut apples. Hence, we propose that the upregulation of genes associated with redox reactions may be due to the abundance of phenolic substances in apple.

Cantaloupe

A higher pH value of cantaloupe may promote flagellar assembly in *L. monocytogenes*. Flagellar plays an important role in biofilm formation, attachment and colonization of fresh produce (Gorski et al., 2003; Lemon et al., 2007). In our study, the flagellar assembly pathway was up-regulated by *L. monocytogenes* on cantaloupe. Consistent with our results, Kang et al. (2019) also showed that flagellar gene expression is critical for *L. monocytogenes* adaptation in the cantaloupe slice environment. Interestingly, *L. monocytogenes* did not show upregulation of flagellar assembly on apple, celery, cucumber, and tomato. The possible explanation is that pH may affect the regulation of flagellar assembly. Compared with the pH in cantaloupe, the pH of the apple, celery, cucumber, and tomato, is lower. Tresse et al.(2006) reported that low pH (e.g., pH=5) may lead to the downregulation of flagellin synthesis compared with pH 7. Hence, we propose that a higher pH of cantaloupe in our study can possibly explain the upregulation of

flagellar assembly on fresh-cut cantaloupe compared with that of *L. monocytogenes* on other fresh produce or in PBS.

The lower levels of phenolics substance possibly facilitates the upregulation of oxidative phosphorylation pathway in *L. monocytogenes* on cantaloupes. We observed that oxidative phosphorylation pathway was up-regulated by *L. monocytogenes* on cantaloupe compared with that of *L. monocytogenes* on apple or celery. This pathway may be affected by total phenolic content on fresh-cut produce. Phenolic compounds can penetrate bacterial membrane, enter the cytosol, quench free electrons from electron transport chain (ETC), and then disrupt proton motive force that is essential for oxidative phosphorylation in which ATP is formed (Apostolidis et al., 2008; Ding et al., 2018; Lin et al., 2005). In previous studies, the total phenolic substances in cantaloupe were the lowest compared with that in celery and apple (Isabelle et al., 2010; Yao & Ren, 2011). Hence, we propose that the upregulation of oxidative phosphorylation pathway is probably due to the lower total phenolic content in cantaloupe.

Celery

In our study, branched chain amino acids (i.e., valine, leucine and isoleucine) (BCAAs) biosynthesis pathway was up-regulated on fresh-cut celery, indicating that *L. monocytogenes* may synthesize branched chain amino acids to facilitate its adaptation on celery. One of the BCAAs, isoleucine, is a fatty acid precursor, which is important for determining the fatty acid profile of *L. monocytogenes* (Zhu et al., 2005). This amino acid can help to increase the amount of anteiso-C15:0 at the expense of anteiso-C17:0, which can make *L. monocytogenes* survive and grow in the cold temperature (Beales et al., 2004; Zhu et al., 2005; Gandhi et al., 2006). The mechanisms of the upregulation of BCAAs pathway are still unclear. Kang et al. (2019) studied BCAAs biosynthesis of *L. monocytogenes* on cantaloupe with the reference of BHI. They

suggested that the upregulation of BCAAs biosynthesis pathway in *L. monocytogenes* may result from insufficient levels of exogenous sources of BCAAs. Interestingly, in our results, according to the USDA nutritional database, the amount of BCAAs in apple is lower than that present in celery and BCAAs biosynthesis pathway showed down-regulation on fresh-cut apples (USDA, 2018a, 2018c). Therefore, inadequate levels of BCAAs in fresh produce may not explain the upregulation of BCAAs biosynthesis pathway in *L. monocytogenes*. BCAAs biosynthesis can also affect pantothenate and CoA production. Our data showed that the pathway of pantothenate and CoA production was also up-regulated on celery. Pantothenate and CoA are essential for synthesizing proteins, carbohydrates, and lipids. One of the precursors for the pantothenate production is 2-ketoisovalerate, which can be synthesized during BCAAs biosynthesis process. (Amorim Franco & Blanchard, 2017).

4.4.4 Summary

In summary, we studied transcriptomic profiles of *L. monocytogenes* on all five fresh-cut produce types by directly harvesting cells from the food matrix and doing RNA sequencing. This study provides a detailed assessment of *L. monocytogenes* transcriptomic landscapes on fresh-cut produce. With the transcriptomic profiles, physiological states of *L. monocytogenes* on fresh produce are well illustrated. For example, we identified a core transcriptome that included commonly up- and down-regulated genes and pathways of *L. monocytogenes* on fresh produce compared to PBS. The core transcriptome can contribute to *L. monocytogenes* adaptation on all fresh produce. This transcriptome involves amino acid metabolism, fatty acid metabolism, carbon metabolism, etc. The information obtained can provide molecular assessment of metabolic and stress responses that may be important for *L. monocytogenes* adaptation on fresh-cut produce. Also, we analyzed *L. monocytogenes* adaptation strategies on specific fresh-cut

produce using pairwise comparison between fresh-cut produce. We found that transcriptomic response of *L. monocytogenes* can be affected by antimicrobials, carbohydrates, amino acids and pH in fresh-cut produce. This study may contribute to providing potential targets (e.g., specific and highly expressed genes of *L. monocytogenes* on all fresh-cut produce) for future detection and control strategies of *L. monocytogenes* on fresh-cut produce.

4.5 Acknowledgements

This study was supported by the Agriculture and Food Research Initiative (AFRI) awards no.2016-67017-24432 from the U.S. Department of Agriculture (USDA) National Institute of Food and Agriculture (NIFA). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Table 4.1 pH values of the fresh-cut produce used in this study[1]

	apple	tomato	cucumber	celery	honeydew	spinach	corn	cantaloupe
pH	3.61	4.36	5.9	5.96	6.1	6.91	7.41	7.43

[1] pH values were the averages of pH of tested fresh produce

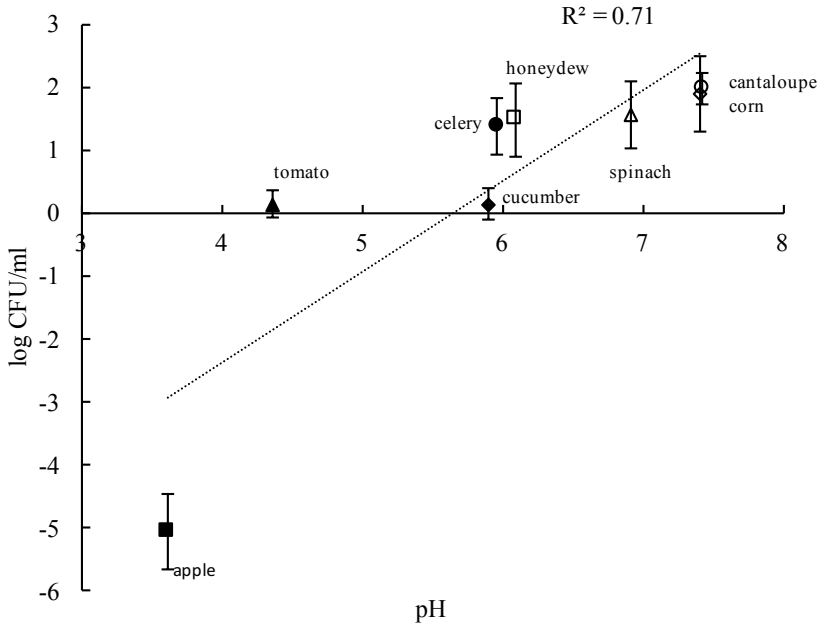

Figure 4.1 Changes of *L. monocytogenes* populations in fresh-cut produce juice at 4 °C for 7 days. The initial inoculum of *L. monocytogenes* was 10^9 CFU/ml.

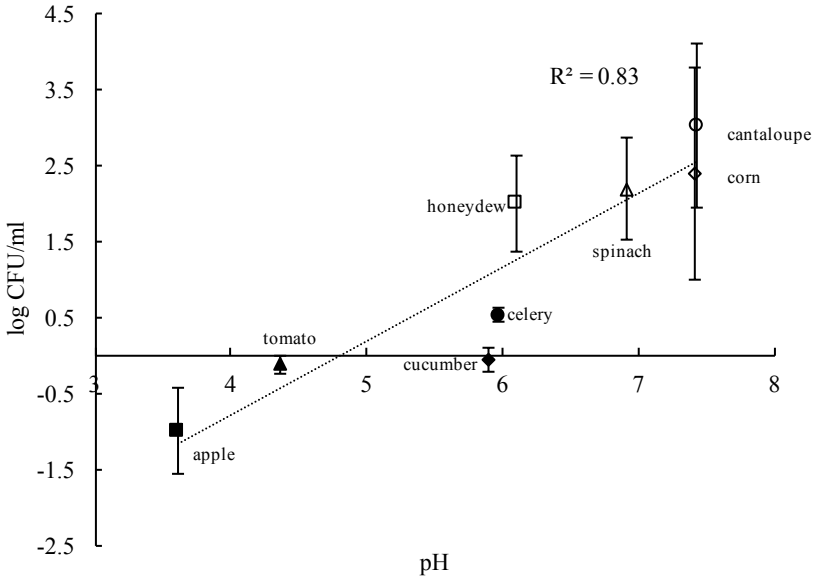

Figure 4.2 Changes of *L. monocytogenes* populations in fresh-cut produce juice at 4°C for 7 days. The initial inoculum of *L. monocytogenes* was 10^5 CFU/ml.

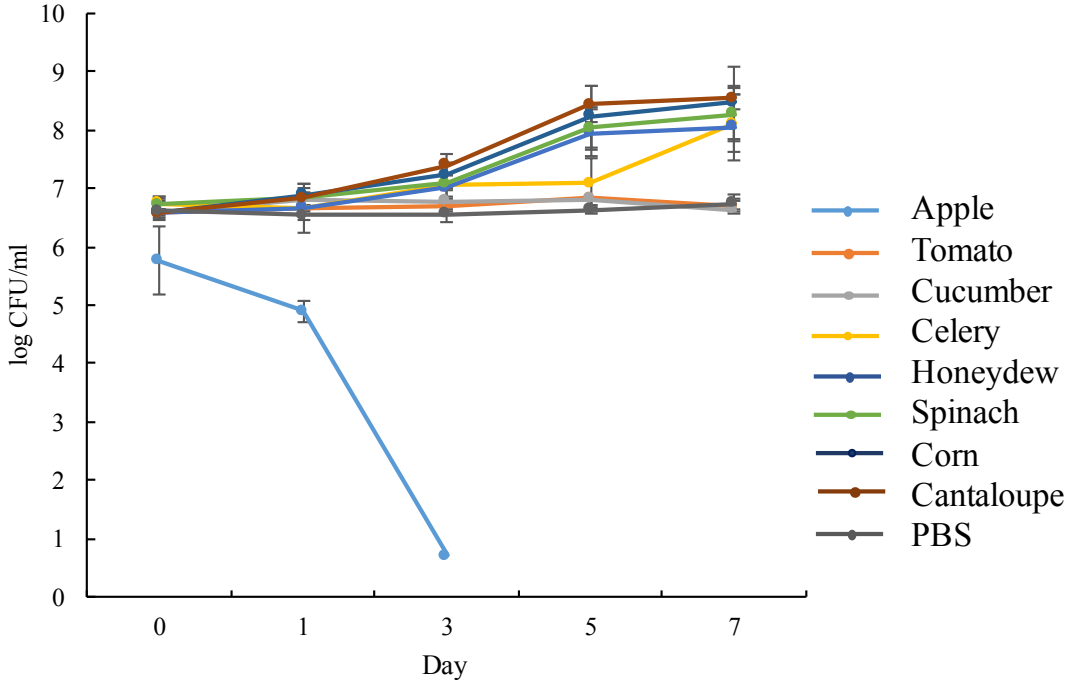

Figure 4.3 *L. monocytogenes* growth and survival in fresh-cut produce juice during 7 days storage at 4°C. The initial inoculum of *L. monocytogenes* was 10^9 CFU/ml.

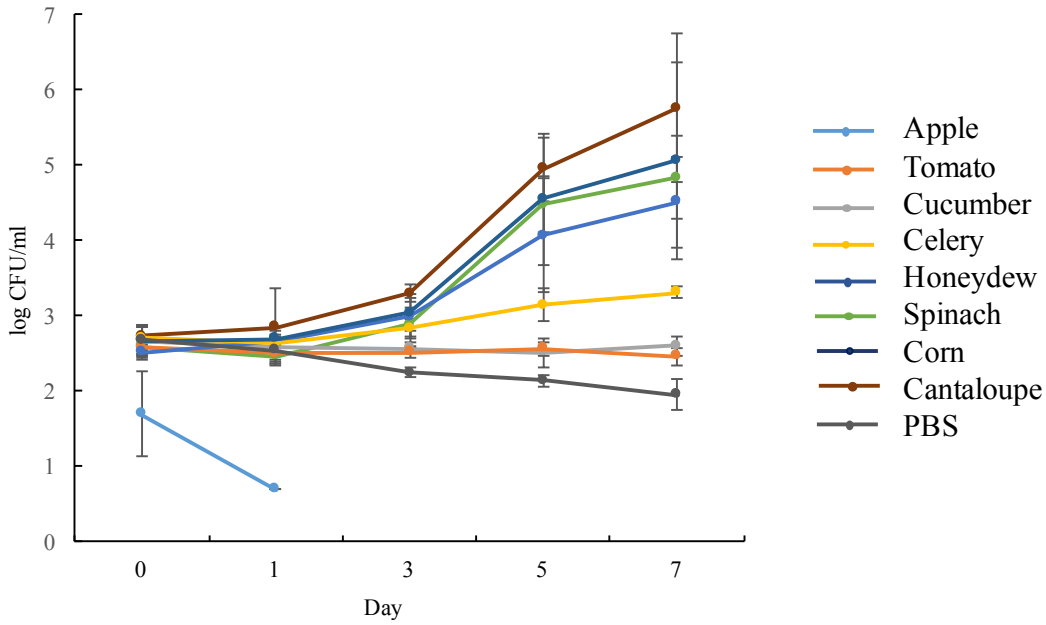

Figure 4.4 *L. monocytogenes* growth and survival in fresh-cut produce juice during 7 days storage at 4 °C. The initial inoculum of *L. monocytogenes* was 10^5 CFU/ml.

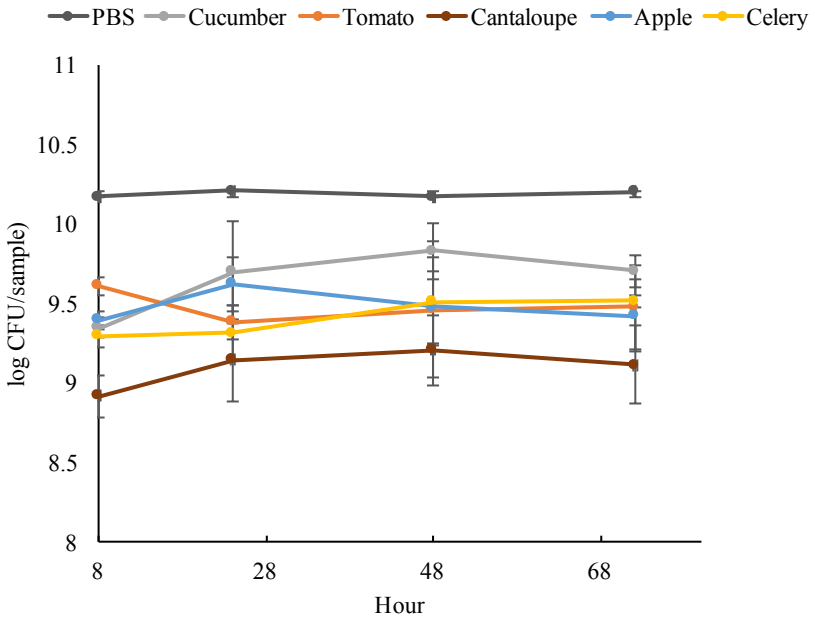

Figure 4.5 *L. monocytogenes* cell populations on fresh-cut produce and in PBS at 4°C for 72 h. *L. monocytogenes* cells were directly harvested from filter membranes sandwiched between two pieces of the same fresh-cut produce. Populations of *L. monocytogenes* cells that were harvested from six pieces of filter membranes were quantified.

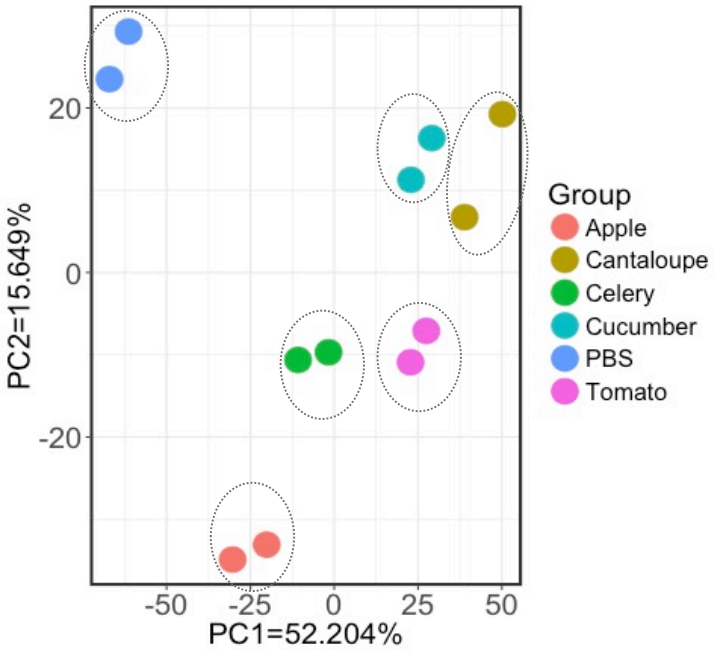

Figure 4.6 Principal component analysis of overall transcriptomes of *L. monocytogenes* on fresh-cut produce and PBS samples. Two replicate samples for each type of produce or PBS were shown with the same color.

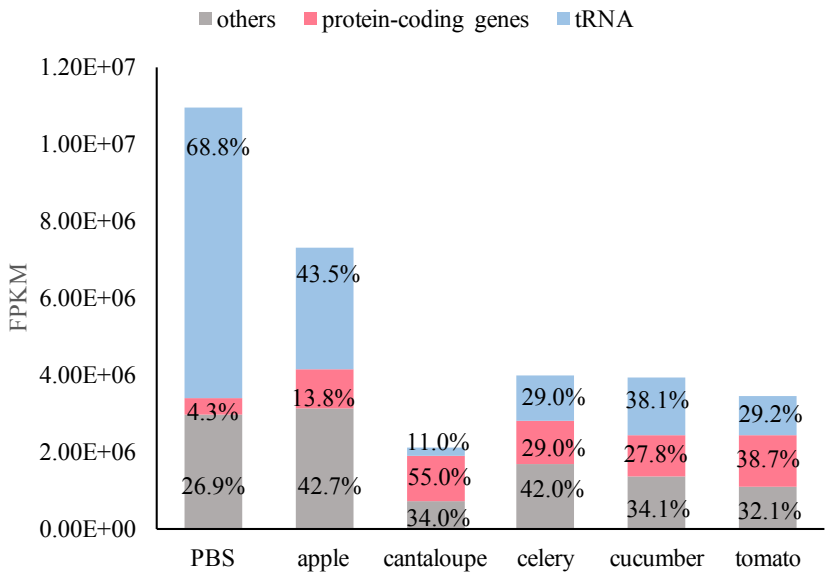

Figure 4.7 Compositions of overall transcriptomes in *L. monocytogenes* on fresh-cut produce and PBS. Overall gene expression level for each sample was shown as the sum of fragments per kilo bases per million reads (FPKM) of each transcript. The percentage of each transcript category in the overall transcriptomes was displayed.

Table 4.2 Highly expressed protein-coding genes in *L. monocytogenes* in PBS[1,2]

Gene product ID	Gene product	PBS
LMOF2365_RS12185	hypothetical protein	89430.20
LMOF2365_RS12300	MULTISPECIES: hypothetical protein	70050.35
LMOF2365_RS11540	hypothetical protein	54091.00
LMOF2365_RS05100	ATP-dependent Clp protease ATP-binding subunit	23958.65
LMOF2365_RS02625	MULTISPECIES: DUF896 domain-containing protein	23691.45
LMOF2365_RS14285	hypothetical protein	14959.78
LMOF2365_RS11280	2,3-bisphosphoglycerate-dependent phosphoglycerate mutase	6939.10
LMOF2365_RS07555	MULTISPECIES: DUF1292 domain-containing protein	6893.97
LMOF2365_RS02065	hypothetical protein	5886.05
LMOF2365_RS04080	hypothetical protein	5665.36
LMOF2365_RS07565	MULTISPECIES: DUF965 domain-containing protein	4261.40
LMOF2365_RS11405	low molecular weight phosphatase family protein	3380.65
LMOF2365_RS12590	MULTISPECIES: ribosomal subunit interface protein	3322.12
LMOF2365_RS07560	Holliday junction resolvase RuvX	3273.94
LMOF2365_RS06130	MULTISPECIES: transcriptional regulator	3269.27
LMOF2365_RS12210	extradiol dioxygenase	3029.58
LMOF2365_RS06560	N-acetyltransferase	3003.42
LMOF2365_RS01055	thiol-activated toxin listeriolysin O	2837.24
LMOF2365_RS02060	MULTISPECIES: hypothetical protein	2761.19
LMOF2365_RS12900	hypothetical protein	2590.97
LMOF2365_RS05435	DUF5068 domain-containing protein	2216.66
LMOF2365_RS11535	MULTISPECIES: type 1 glutamine amidotransferase	1981.48
LMOF2365_RS11205	adaptor protein MecA	1963.74
LMOF2365_RS14095	XRE family transcriptional regulator	1874.33
LMOF2365_RS06570	cell division suppressor protein YneA	1846.95
LMOF2365_RS02005	hypothetical protein	1754.63
LMOF2365_RS00990	MULTISPECIES: hypothetical protein	1634.63
LMOF2365_RS12375	ATP-dependent Clp protease proteolytic subunit	1593.99

[1] Gene transcript levels were quantified as fragments per kilo bases per million reads (FPKM)
[2] Gene transcript levels that ranked top 28 (1% of protein-coding genes) in PBS were considered as highly expressed and displayed

Table 4.3 Highly transcribed protein-coding genes in *L. monocytogenes* on fresh-cut produce[1,2]

Gene product ID	Gene product	Apple	Cantaloupe	Celery	Cucumber	Tomato
LMOF2365_RS12300	MULTISPECIES: hypothetical protein	55415.50	27336.50	77746.60	61666.40	61130.30
LMOF2365_RS11540	hypothetical protein	52315.45	11142.05	24911.65	22013.20	46135.00
LMOF2365_RS06870	MULTISPECIES: cold-shock protein CspA	36699.15	12661.80	34290.65	16192.30	35952.25
LMOF2365_RS12185	hypothetical protein	18988.80	6726.52	15985.15	7476.80	21665.70
LMOF2365_RS02575	MULTISPECIES: 50S ribosomal protein L32	15829.15	15404.35	5978.80	10927.05	35501.30
LMOF2365_RS00990	MULTISPECIES: hypothetical protein	15826.10	5800.69	13138.40	8002.72	15630.55
LMOF2365_RS11210	MULTISPECIES: transcriptional regulator Spx	14913.85	5413.61	6780.13	4368.80	8855.08
LMOF2365_RS12865	hypothetical protein	13669.55	10052.91	21292.40	6935.85	8812.93
LMOF2365_RS11405	low molecular weight phosphatase family protein	11850.67	5728.77	11238.40	9898.73	11544.35

[1] Gene transcript levels of *L. monocytogenes* all fresh-cut produce were quantified as fragments per kilo bases per million reads (FPKM).

[2] Gene transcript levels that ranked top 28 (1 % of protein coding genes) on all fresh-cut produce were considered as highly expressed and displayed.

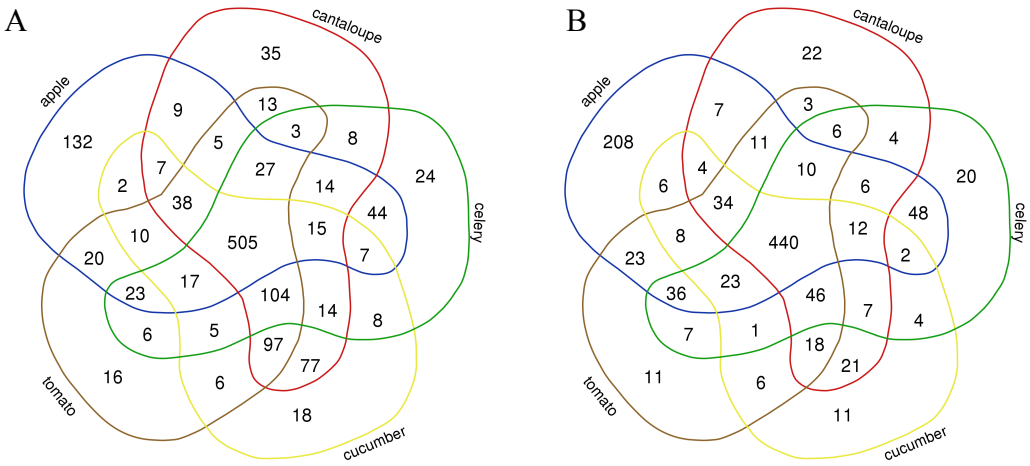

Figure 4.8 Differential gene expression of *L. monocytogenes* on fresh-cut produce. A. Commonly and specifically up-regulated genes ($\log_2$ FC ≥1). B. Commonly and specifically down-regulated genes ($\log_2$ FC ≤-1). Fold change of gene expression was calculated in comparison with that of *L. monocytogenes* cells in PBS. The numbers shown in this figure were numbers of the up-/down-regulated genes.

Table 4.4 List of pathways that showed significant up- and down-regulation ($P < 0.05$, FDR < 0.25) in *L. monocytogenes* on all fresh-cut produce compared to pathways in *L. monocytogenes* in PBS

Up-regulated	Down-regulated
Cysteine and methionine metabolism	Phosphotransferase system (PTS)
2-Oxocarboxylic acid metabolism	Starch and sucrose metabolism
Fatty acid metabolism	Carbon metabolism
ABC transporters	Pentose and glucuronate interconversions
Fatty acid biosynthesis	Pentose phosphate pathway
Lysine biosynthesis	Glyoxylate and dicarboxylate metabolism
Histidine metabolism	Glycolysis/Gluconeogenesis
	Microbial metabolism in diverse environment

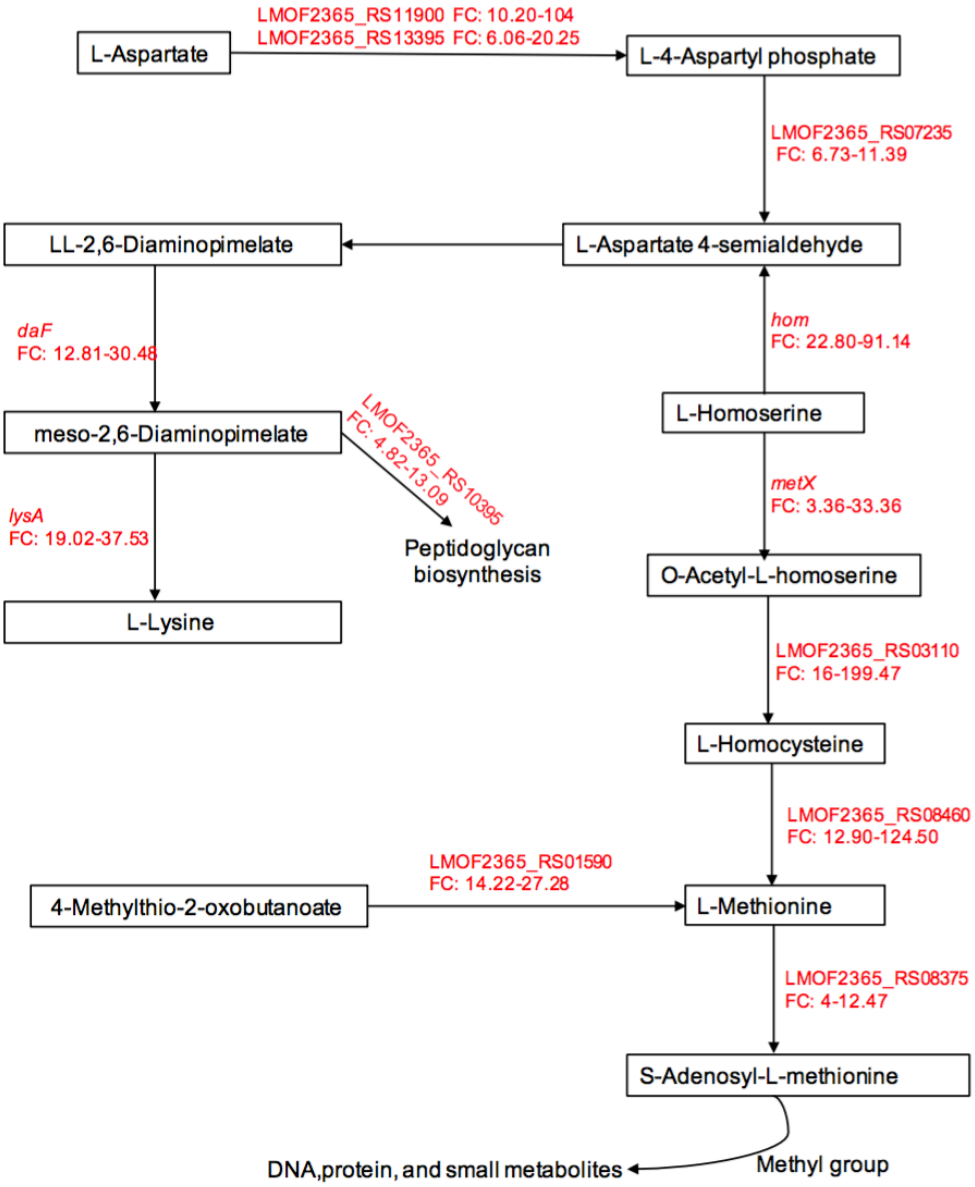

Figure 4.9 Cysteine and methionine metabolism and lysine biosynthesis pathways in *L. monocytogenes*. The pathway diagram was built based on the information acquired from KEGG database (Kanehisa et al., 2000). Genes with their fold changes were marked in red. These genes were commonly up-regulated on fresh-cut produce as compared to PBS. The minimal and maximum values of differential gene expression (FC $\geq$ 2, FDR < 0.05, P < 0.05) for each gene in *L. monocytogenes* on fresh-cut produce were displayed.

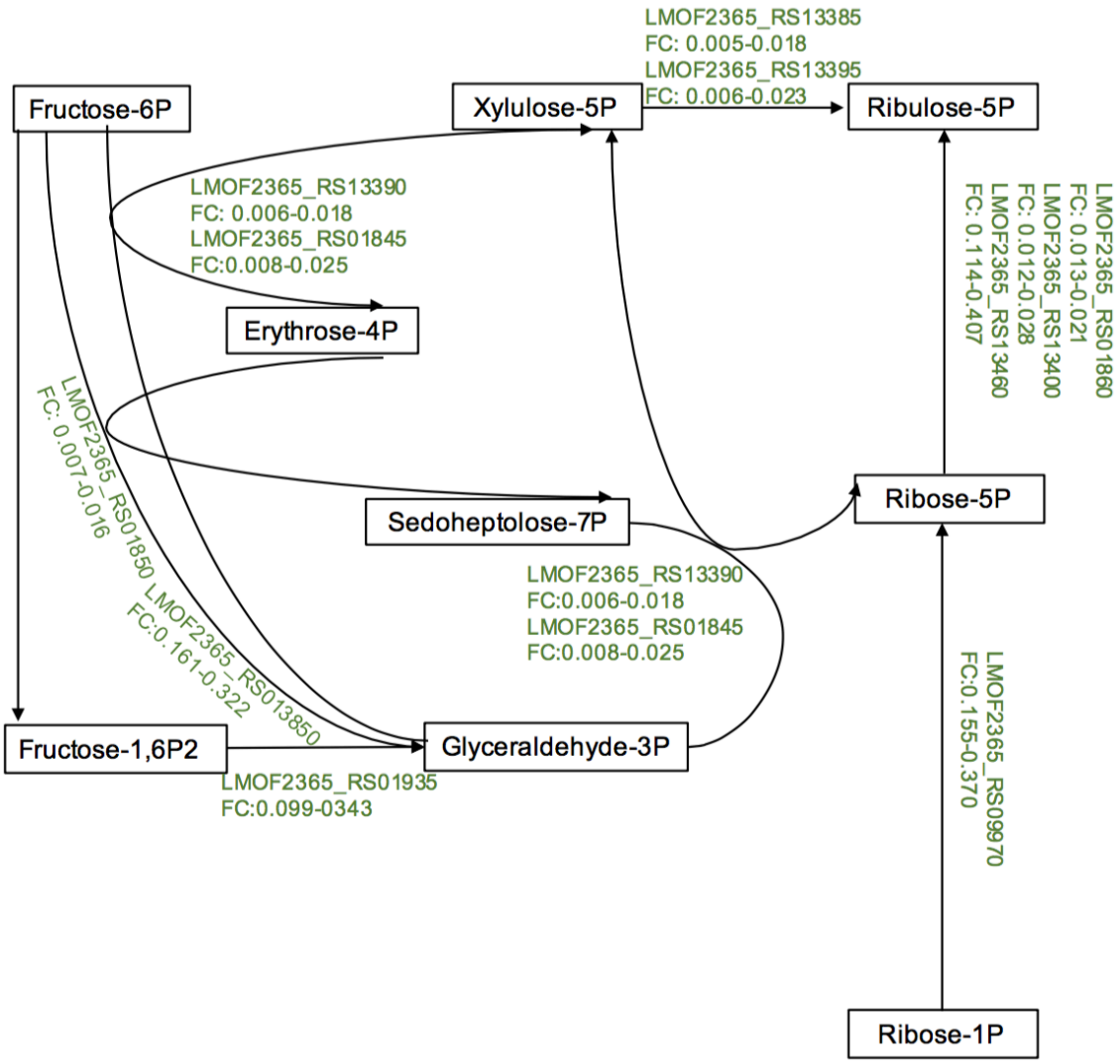

Figure 4.10 Pentose phosphate pathways in *L. monocytogenes*. The pathway diagram was made according to the KEGG database (Kanehisa et al., 2000). Genes with their fold changes were marked in green. These genes were commonly down-regulated on fresh-cut produce as compared to PBS. The minimal and maximum values of differential gene expression (FC ≤0.5, FDR < 0.05, $P < 0.05$) for each gene in *L. monocytogenes* on fresh-cut produce were displayed.

Table 4.5 List of genes that were only expressed on fresh-cut produce[1]

Gene ID	Apple	Cantaloupe	Celery	Cucumber	Tomato	Gene product
LMOF2365_RS00645	60.81[2]	96.35	35.86	50.66	58.35	EAL domain-containing protein
LMOF2365_RS02800	124.46	132.26	65.17	65.97	48.71	MULTISPECIES: ACT domain-containing protein
LMOF2365_RS03210	202.27	152.89	109.82	89.84	100.28	hypothetical protein
LMOF2365_RS03835	67.85	91.52	70.71	53.42	97.28	Crp/Fnr family transcriptional regulator
LMOF2365_RS03865	59.65	43.43	40.58	20.82	52.39	pseudogene
LMOF2365_RS04110	25.41	137.02	22.20	86.57	113.72	ABC transporter ATP-binding protein
LMOF2365_RS04135	39.94	29.50	27.18	31.26	33.39	bifunctional (p)ppGpp synthetase/guanosine-3\',5\'-bis(diphosphate) 3\'-pyrophosphohydrolase
LMOF2365_RS04175	26.25	28.34	16.59	47.75	66.00	VOC family protein
LMOF2365_RS04425	73.96	160.66	64.20	69.22	122.26	transcriptional regulator
LMOF2365_RS04985	37.42	412.40	140.43	28.82	842.34	teichoic acid D-Ala incorporation-associated protein DltX
LMOF2365_RS05060	115.84	169.80	170.57	84.54	277.29	MarR family transcriptional regulator
LMOF2365_RS08005	113.46	258.10	74.82	294.10	51.81	N-acetyl-gamma-glutamyl-phosphate reductase
LMOF2365_RS08175	259.20	141.04	157.89	88.44	132.79	hypothetical protein
LMOF2365_RS09050	1174.23	131.33	113.20	198.64	162.68	5-(carboxyamino)imidazole ribonucleotide mutase
LMOF2365_RS10075	20.38	101.53	48.83	42.37	60.59	N-acetyltransferase
LMOF2365_RS10090	24.66	269.33	949.08	73.84	48.45	MULTISPECIES: acetolactate synthase small subunit
LMOF2365_RS10520	82.61	112.04	48.12	39.33	29.71	hypothetical protein
LMOF2365_RS10560	147.52	206.22	79.37	134.53	256.55	DUF4305 domain-containing protein

LMOF2365_RS10995	200.99	177.78	158.75	86.05	227.31	nucleotide pyrophosphohydrolase
LMOF2365_RS11445	44.89	40.73	14.89	28.67	24.80	MFS transporter
LMOF2365_RS12265	152.06	50.95	192.49	28.72	83.58	transcriptional activator
LMOF2365_RS12360	32.96	80.52	23.35	71.76	132.91	hypothetical protein
LMOF2365_RS14705	138.85	292.22	23.00	194.31	295.75	hypothetical protein
LMOF2365_RS14745	137.55	319.08	155.07	112.37	668.19	hypothetical protein

[1] The number shown in each cell is the average fragments per kilo bases per million reads (FPKM) of each gene in *L. monocytogenes* on fresh-cut produce

[2] Transcript levels of genes (FPKM) that are more than 20 in *L. monocytogenes* were displayed.

Table 4.6 List of pathways that showed significant up- and down-regulation ($P < 0.05$, FDR < 0.25) in *L. monocytogenes* on specific fresh-cut produce compared to those in *L. monocytogenes* in PBS

	Up-regulated	Down-regulated
Apple	------	Aminoacyl-tRNA biosynthesis Mismatch repair Cationic antimicrobial peptide (CAMP) resistance
Cantaloupe	Beta-lactam resistance Peptidoglycan biosynthesis	------
	Flagellar assembly	------
Celery	Arginine and proline metabolism	
Cucumber	------	------
Tomato	Terpenoid backbone biosynthesis	Methane metabolism

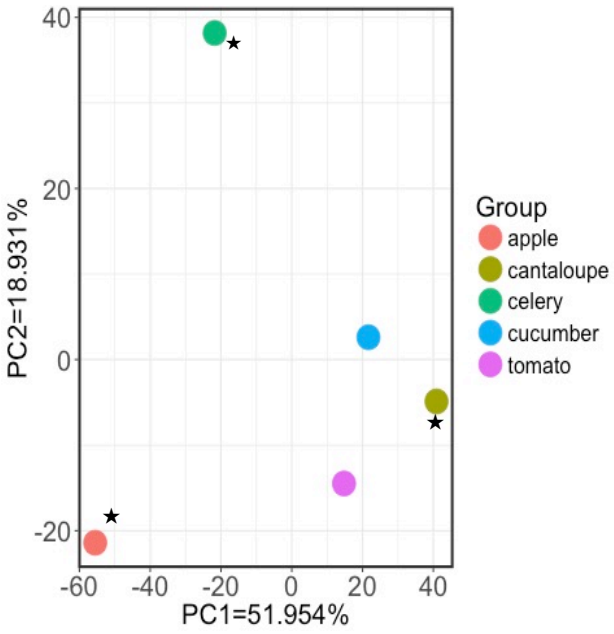

Figure 4.11 Principal component analysis of overall gene expression in *L. monocytogenes* on fresh-cut produce. Each dot showed the average *L. monocytogenes* transcriptome in two replicate samples. The average *L. monocytogenes* transcriptomes were quantified using the average fragments per kilo bases per million reads (FPKM) between two replicate samples.

Table 4.7 List of significantly up-/down-regulated pathways ($P < 0.05$, FDR < 0.25) in *L. monocytogenes* on fresh-cut produce during pairwise comparison

Fresh-cut produce	Pathways
Cantaloupe[1] (Up-regulated)	Ribosome
	Flagellar assembly
	Bacterial chemotaxis
	Aminoacyl-tRNA biosynthesis
	Purine metabolism
	Oxidative phosphorylation
	Peptidoglycan biosynthesis
	Metabolic pathways
	Mismatch repair
	One carbon pool by folate
Celery[2] (Up-regulated)	Phosphotransferase system (PTS)
	Starch and sucrose metabolism
	Valine, leucine and isoleucine biosynthesis
	Amino sugar and nucleotide sugar metabolism
	Pantothenate and CoA biosynthesis
Apple[3] (Down-regulated)	Ribosome
	Pyrimidine metabolism
	Mismatch repair
	C5-Branched dibasic acid metabolism
	Peptidoglycan biosynthesis
	Aminoacyl-tRNA biosynthesis
	Cationic antimicrobial peptide (CAMP) resistance

[1] The pathways were up-regulated on fresh-cut cantaloupes versus apples and celery
[2] The pathways were up-regulated on fresh-cut celery versus apples and cantaloupes
[3] The pathways were down-regulated on fresh-cut apples versus celery and cantaloupes

Table 4.8 Nutrient concentration and pH value of fresh produce used in transcriptomic profiling of *L. monocytogenes*

	Unit	Apple	Cantaloupe	Celery	Cucumber	Tomato
Carbohydrates[1]	g/100g	13.81	8.16	2.97	3.63	3.89
Amino acids[1]	g/100g	0.201	0.754	0.495	0.539	0.865
Fatty acids[1]	g/100g	0.086	0.135	0.153	0.074	0.142
Glucose[1]	g/100g	2.43	1.54	0.40	0.76	1.25
Valine[1]	g/100g	0.012	0.033	0.027	0.022	0.018
Isoleucine[1]	g/100g	0.006	0.021	0.021	0.021	0.028
Leucine[1]	g/100g	0.013	0.029	0.032	0.029	0.025
Lysine[1]	g/100g	0.012	0.030	0.027	0.029	0.027
Methionine[1]	g/100g	0.001	0.012	0.005	0.006	0.006
Cysteine[1]	g/100g	0.001	0.002	0.004	0.004	0.009
Fe, iron[1]	mg/100g	0.12	0.21	0.20	0.28	0.27
Total phenolic[2]	mg/GAE/g	1.25	0.11	0.30	0.35	0.14
pH	---	3.61	7.43	5.96	5.90	4.36

[1] Data obtained from USDA website (USDA, 2018a, 2018b, 2018c, 2018d, 2018e)
[2] Data obtained from Dewanto et al. (2002), Athunibat et al. (2009), USDA (2018d), Isabelle et al. (2010), and Yao & Ren (2011)

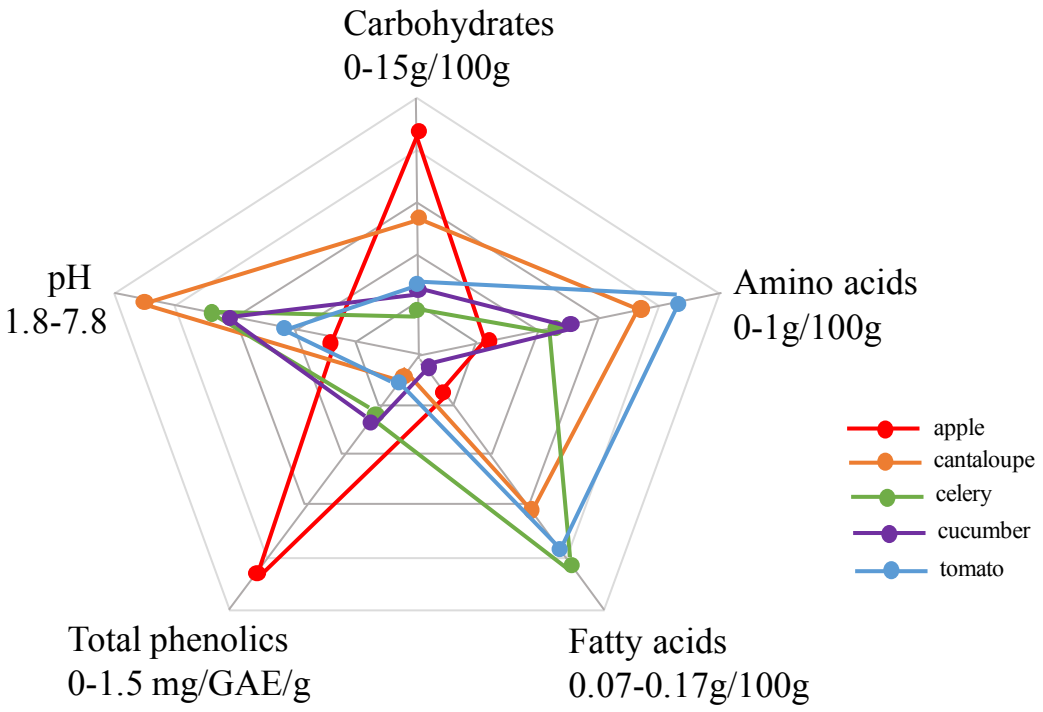

Figure 4.12 Nutrient concentration and pH levels on all fresh-cut produce. In this radar map, the amount of carbohydrate ranges from 0g/100g to 15g/100g, each quinquesection stands for 3 g/100g. The amount of amino acids ranges from 0g/100g to 1g /100g, each quinquesection stands for 0.2g/100g. The amount of fatty acids ranges from 0.07g/100g to 0.17g/100g, each quinquesection stands for 0.02 g/100g. The amount of total phenolics ranges from 0mg/GAE/g to 1.5mg/GAE/g, each quinquesection stands for 0.3 mg/GAE/g. pH value ranges from 1.8 to 7.8, each quinquesection stands for 1.2. Then the nutrient concentration and pH value in all fresh-cut produce were displayed in redar map according to the data obtained from USDA National Nutrient Database for Standard Reference as well as Dewanto et al. (2002), Athunibat et al. (2009), Isabelle et al. (2010) and Yao & Ren (2011).

References

Ackermann, J., Fischer, M., & Amado, R. (1992). Changes in sugars, acids, and amino acids during ripening and storage of apples (cv. Glockenapfel). *Journal of Agricultural and Food Chemistry, 40*(7), 1131-1134.

Alifano, P., Fani, R., Liò, P., Lazcano, A., Bazzicalupo, M., Carlomagno, M. S., & Bruni, C. B. (1996). Histidine biosynthetic pathway and genes: structure, regulation, and evolution. *Microbiological Reviews, 60*(1), 44.

Althunibat, O. Y., Hashim, R. B., Taher, M., Daud, J. M., Ikeda, M. A., & Zali, B. (2009). In vitro antioxidant and antiproliferative activities of three Malaysian sea cucumber species. *European Journal of Scientific Research, 37*(3), 376-387.

Amorim Franco, T. M., & Blanchard, J. S. (2017). Bacterial branched-chain amino acid biosynthesis: structures, mechanisms, and drugability. *Biochemistry, 56*(44), 5849-5865.

Anders, S., Pyl, P. T., & Huber, W. (2015). HTSeq-a Python framework to work with high-throughput sequencing data. *Bioinformatics, 31*(2), 166-169.

Apostolidis, E., Kwon, Y. I., & Shetty, K. (2008). Inhibition of *Listeria monocytogenes* by oregano, cranberry and sodium lactate combination in broth and cooked ground beef systems and likely mode of action through proline metabolism. *International Journal of Food Microbiology, 128*(2), 317-324.

Babic, I., Watada, A., & Buta, J. (1997). Growth of *Listeria monocytogenes* restricted by native microorganisms and other properties of fresh-cut spinach. *Journal of Food Protection, 60*(8), 912-917.

Beales, N. (2004). Adaptation of microorganisms to cold temperatures, weak acid preservatives, low pH, and osmotic stress: a review. *Comprehensive Reviews in Food Science and Food Safety, 3*(1), 1-20.

Bhunia, A. K. (2018). *Foodborne microbial pathogens: mechanisms and pathogenesis*: Springer.

Böckmann, R., Dickneite, C., Middendorf, B., Goebel, W., & Sokolovic, Z. (1996). Specific binding of the *Listeria monocytogenes* transcriptional regulator PrfA to target sequences requires additional factor (s) and is influenced by iron. *Molecular Microbiology, 22*(4), 643-653.

Bolger, A. M., Lohse, M., & Usadel, B. (2014). Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics, 30*(15), 2114-2120.

CDC. (2012). Multistate outbreak of listeriosis linked to whole cantaloupes from Jensen Farms, Colorado (final update). *Available at: https://www.cdc.gov/listeria/outbreaks/cantaloupes-jensen-farms/index.html. Accessed on August 14th 2018.*

CDC. (2015). Multistate outbreak of listeriosis linked to commercially produced, prepackaged caramel apples made from Bidart Bros. Apples (final update). *Available at: https://www.cdc.gov/listeria/outbreaks/caramel-apples-12-14/index.html Accessed on December 26th 2018.*

CDC. (2018). *Listeria* (Listeriosis). *Available at: https://www.cdc.gov/listeria/ Accessed on December 3rd 2018.*

Chiang, P. K., Gordon, R. K., Tal, J., Zeng, G., Doctor, B., Pardhasaradhi, K., & McCann, P. P. (1996). S-Adenosylmethionine and methylation. *The FASEB Journal, 10*(4), 471- 480.

Conway, W. S., Leverentz, B., Saftner, R. A., Janisiewicz, W. J., Sams, C. E., & Leblanc, E. (2000). Survival and growth of *Listeria monocytogenes* on fresh-cut apple slices and its interaction with Glomerella cingulata and Penicillium expansum. *Plant Disease, 84*(2), 177-181.

Crucello, A., Furtado, M. M., Chaves, M. D., & Sant'Ana, A. S. (2019). Transcriptome sequencing reveals genes and adaptation pathways in *Salmonella* Typhimurium inoculated in four low water activity foods. *Food Microbiology*, *82*, 426-435.

Danyluk, M. D., Friedrich, L. M., & Schaffner, D. W. (2014). Modeling the growth of *Listeria monocytogenes* on cut cantaloupe, honeydew and watermelon. *Food Microbiology, 38*, 52-55.

Datta, A., & Kothary, M. H. (1993). Effects of glucose, growth temperature, and pH on listeriolysin O production in *Listeria monocytogenes*. *Applied and Environmental Microbiology, 59*(10), 3495-3497.

Deng, X., Li, Z., & Zhang, W. (2012). Transcriptome sequencing of *Salmonella enterica* serovar Enteritidis under desiccation and starvation stress in peanut oil. *Food Microbiology, 30*(1), 311-315.

Dewanto, V., Wu, X., Adom, K. K., & Liu, R. H. (2002). Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. *Journal of Agricultural and Food Chemistry, 50*(10), 3010-3014.

Ding, L., Xiao, S., Liu, D., & Pang, W. (2018). Effect of dihydromyricetin on proline metabolism of *Vibrio parahaemolyticus*: Inhibitory mechanism and interaction with molecular docking simulation. *Journal of Food Biochemistry, 42*(1), e12463.

Dogovski, C., Atkinson, S. C., Dommaraju, S. R., Hor, L., Dobson, R., Hutton, C., Gerrard, J. A., & Perugini, M. (2009). Lysine biosynthesis in bacteria: an unchartered pathway for novel antibiotic design. *Encyclopedia of Life Support Systems, 11*, 116-136.

Doumith, M., Buchrieser, C., Glaser, P., Jacquet, C., Martin, P., 2004. Differentiation of the major *Listeria monocytogenes* serovars by multiplex PCR. *Journal of Clinical Microbiology,* 42, 3819-3822.

Fang, T., Liu, Y., & Huang, L. (2013). Growth kinetics of *Listeria monocytogenes* and spoilage microorganisms in fresh-cut cantaloupe. *Food Microbiology, 34*(1), 174-181.

FDA (2017). Food Code. *Available at:* https://www.fda.gov/Food/GuidanceRegulation/RetailFoodProtection/FoodCode/ucm595139.htm *Accessed on November 16th 2018.*

Fenlon, D. R. (1999). *Listeria monocytogenes* in the natural environment. *FOOD SCIENCE AND TECHNOLOGY-NEW YORK-MARCEL DEKKER-*, 21-38.

Ferla, M. P., & Patrick, W. M. (2014). Bacterial methionine biosynthesis. *Microbiology, 160*(8), 1571-1584.

Fink, R. C., Black, E., Hou, Z., Sugawara, M., Sadowsky, M. J., & Diez-Gonzalez, F. (2012). Transcriptional responses of *Escherichia coli* K12 and O157: H7 in association with lettuce leaves. *Applied and Environmental Microbiology*, 07454-07411.

FSAI. (2005). The control and management of *Listeria monocytogenes* contamination of food. *Food Safety Authority of Ireland: Dublin, Ireland*.

Gandhi, M., & Chikindas, M. L. (2007). *Listeria*: a foodborne pathogen that knows how to survive. *International Journal of Food Microbiology, 113*, 1-15.

Gaul, L. K., Farag, N. H., Shim, T., Kingsley, M. A., Silk, B. J., & Hyytia-Trees, E. (2012). Hospital-acquired listeriosis outbreak caused by contaminated diced celery-Texas, 2010. *Clinical Infectious Diseases, 56*(1), 20-26.

Gilbreth, S. E., Benson, A. K., & Hutkins, R. W. (2004). Catabolite repression and virulence gene expression in *Listeria monocytogenes*. *Current Microbiology, 49*(2), 95-98.

Goudeau, D. M., Parker, C. T., Zhou, Y., Sela, S., Kroupitski, Y., & Brandl, M. T. (2013). The *Salmonella* transcriptome in lettuce and cilantro soft rot reveals a niche overlap with the animal host intestine. *Applied and Environmental Microbiology, 79*(1), 250-262.

Gorski, L., Palumbo, J. D., & Mandrell, R. E. (2003). Attachment of *Listeria monocytogenes* to radish tissue is dependent upon temperature and flagellar motility. *Applied and Environmental Microbiology, 69*(1), 258-266.

Isabelle, M., Lee, B. L., Lim, M. T., Koh, W.-P., Huang, D., & Ong, C. N. (2010). Antioxidant activity and profiles of common fruits in Singapore. *Food Chemistry, 123*(1), 77-84.

Jaradat, Z. W., & Bhunia, A. K. (2002). Glucose and nutrient concentrations affect the expression of a 104-kilodalton *Listeria* adhesion protein in *Listeria monocytogenes*. *Applied and Environmental Microbiology, 68*(10), 4876-4883.

Junttila, J. R., Niemelä, S., & Hirn, J. (1988). Minimum growth temperatures of *Listeria monocytogenes* and non-haemolytic *listeria*. *Journal of Applied Bacteriology, 65*(4), 321-327.

Kajfasz, J. K., Rivera-Ramos, I., Abranches, J., Martinez, A. R., Rosalen, P. L., Derr, A. M., Quivey, R. G., & Lemos, J. A. (2010). Two Spx proteins modulate stress tolerance, survival, and virulence in *Streptococcus mutans*. *Journal of Bacteriology, 192*(10), 2546-2556.

Kanehisa, M., & Goto, S. (2000). KEGG: kyoto encyclopedia of genes and genomes. *Nucleic Acids Research, 28*(1), 27-30.

Kang, J., Burall, L., Mammel, M. K., & Datta, A. R. (2019). Global transcriptomic response of *Listeria monocytogenes* during growth on cantaloupe slices. *Food Microbiology, 77*, 192-201.

Kim, D., Pertea, G., Trapnell, C., Pimentel, H., Kelley, R., & Salzberg, S. L. (2013). TopHat2: accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions. *Genome Biology, 14*(4), R36.

Lamikanra, O., Chen, J., Banks, D., & Hunter, P. (2000). Biochemical and microbial changes during the storage of minimally processed cantaloupe. *Journal of Agricultural and Food Chemistry, 48*(12), 5955-5961.

Lemon, K. P., Higgins, D. E., & Kolter, R. (2007). Flagellar motility is critical for *Listeria monocytogenes* biofilm formation. *Journal of Bacteriology, 189*(12), 4418-4424.

Lin, Y., Kwon, Y., Labbe, R., & Shetty, K. (2005). Inhibition of *Helicobacter pylori* and associated urease by oregano and cranberry phytochemical synergies. *Applied and Environmental Microbiology, 71*(12), 8558-8564.

Liu, Y., & Ream, A. (2008). Gene expression profiling of *Listeria monocytogenes* strain F2365 during growth in ultrahigh-temperature-processed skim milk. *Applied and Environmental Microbiology, 74*(22), 6859-6866.

Lungu, B., Saldivar, J. C., Story, R., Ricke, S. C., & Johnson, M. G. (2010). The combination of energy-dependent internal adaptation mechanisms and external factors enables *Listeria monocytogenes* to express a strong starvation survival response during multiple-nutrient starvation. *Foodborne Pathogens and Disease, 7*(5), 499-505.

Luo, W., Friedman, M. S., Shedden, K., Hankenson, K. D., & Woolf, P. J. (2009). GAGE: generally applicable gene set enrichment for pathway analysis. *BMC Bioinformatics, 10*(1), 161.

Mutz, K.-O., Heilkenbrinker, A., Lönne, M., Walter, J.-G., & Stahl, F. (2013). Transcriptome analysis using next-generation sequencing. *Current Opinion in Biotechnology, 24*(1), 22-30.

Park, K., Jeong, H., Tanum, J., Yoo, J.-C., & Hong, J. (2019). Poly-l-lysine/poly-l-glutamic acid-based layer-by-layer self-assembled multilayer film for nitric oxide gas delivery. *Journal of Industrial and Engineering Chemistry, 69*, 263-268.

Penteado, A. L., & Leitao, M. F. (2004). Growth of *Listeria monocytogenes* in melon, watermelon and papaya pulps. *International Journal of Food Microbiology, 92*(1), 89-94.

Pratta, G., Zorzoli, R., Boggio, S., Picardi, L., & Valle, E. (2004). Glutamine and glutamate levels and related metabolizing enzymes in tomato fruits with different shelf-life. *Scientia Horticulturae, 100*(1-4), 341-347.

Raina, M., & Ibba, M. (2014). tRNAs as regulators of biological processes. *Frontiers in Genetics, 5*, 171.

Reddy, S., Akgul, A., Karsi, A., Abdelhamed, H., Wills, R. W., & Lawrence, M. L. (2016). The role of *Listeria monocytogenes* cell wall surface anchor protein LapB in virulence, adherence, and intracellular replication. *Microbial Pathogenesis, 92*, 19-25.

Ripio, M.-T., Brehm, K., Lara, M., Suarez, M., & Vazquez-Boland, J.-A. (1997). Glucose-1-phosphate utilization by *Listeria monocytogenes* is PrfA dependent and coordinately expressed with virulence factors. *Journal of Bacteriology, 179*(22), 7174-7180.

Robinson, M. D., McCarthy, D. J., & Smyth, G. K. (2010). edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics, 26*(1), 139-140.

Roosta, H. R., Sajjadinia, A., Rahimi, A., & Schjoerring, J. K. (2009). Responses of cucumber plant to $NH4^+$ and $NO3^-$ nutrition: the relative addition rate technique vs. cultivation at constant nitrogen concentration. *Scientia Horticulturae, 121*(4), 397-403.

Salazar, J. K., Sahu, S. N., Hildebrandt, I. M., Zhang, L., Qi, Y., Liggans, G., Datta, A. R., & Tortorello, M. L. (2017). Growth kinetics of *Listeria monocytogenes* in cut produce. *Journal of Food Protection, 80*(8), 1328-1336.

Salazar, J. K., Wu, Z., Yang, W., Freitag, N. E., Tortorello, M. L., Wang, H., & Zhang, W. (2013). Roles of a novel Crp/Fnr family transcription factor Lmo0753 in soil survival, biofilm production and surface attachment to fresh produce of *Listeria monocytogenes*. *PLoS One, 8*(9), e75736.

Schneider, T., Butz, P., Ludwig, H., & Tauscher, B. (2003). Pressure-induced formation of pyroglutamic acid from glutamine in neutral and alkaline solutions. *LWT-Food Science and Technology, 36*(3), 365-367.

Shimizu, K. (2014). Regulation systems of bacteria such as *Escherichia coli* in response to nutrient limitation and environmental stresses. *Metabolites, 4*(1), 1-35.

Slaghuis, J., Joseph, B., & Goebel, W. (2007). Metabolism and Physiology of *Listeria monocytogenes*. *Listeria monocytogenes: Pathogenesis and Host Response*, 63-80.

Stoll, R., & Goebel, W. (2010). The major PEP-phosphotransferase systems (PTSs) for glucose, mannose and cellobiose of *Listeria monocytogenes*, and their significance for extra-and intracellular growth. *Microbiology, 156*(4), 1069-1083.

Subramanian, A., Tamayo, P., Mootha, V. K., Mukherjee, S., Ebert, B. L., Gillette, M. A., Paulovich, A., Pomeroy, S. L., Golub, T. R., & Lander, E. S. (2005). Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proceedings of the National Academy of Sciences, 102*(43), 15545-15550.

Suo, Y., Gao, S., Baranzoni, G. M., Xie, Y., & Liu, Y. (2018). Comparative transcriptome RNA-Seq analysis of *Listeria monocytogenes* with sodium lactate adaptation. *Food Control, 91*, 193-201.

Tang, S., Orsi, R. H., den Bakker, H. C., Wiedmann, M., Boor, K. J., & Bergholz, T. M. (2015). Transcriptomic analysis of the adaptation of *Listeria monocytogenes* to growth on vacuum-packed cold smoked salmon. *Applied and Environmental Microbiology, 81*(19), 6812-6824.

Trapnell, C., Roberts, A., Goff, L., Pertea, G., Kim, D., Kelley, D. R., Pimentel, H., Salzberg, S. L., Rinn, J. L., & Pachter, L. (2012). Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks. *Nature Protocols, 7*(3), 562.

Tresse, O., Lebret, V., Benezech, T., & Faille, C. (2006). Comparative evaluation of adhesion, surface properties, and surface protein composition of *Listeria monocytogenes* strains after cultivation at constant pH of 5 and 7. *Journal of Applied Microbiology, 101*(1), 53-62.

USDA. (2018a). USDA National Nutrient Database for Standard Reference: Basic report: 09003, apples, raw, with skin. *Availble at: https://ndb.nal.usda.gov/ndb/foods/show/09003?fgcd=&manu=&format=&count=&max=25&offset=&sort=default&order=asc&qlookup=Apples%2C+raw%2C+with+skin+%*

28Includes+foods+for+USDA%27s+Food+Distribution+Program%29&ds=&qt=&qp=&qa=&qn=&q=&ing= Accessed on May 27th, 2019.

USDA. (2018b). USDA National Nutrient Database for Standard Reference: Basic report: 09181, melons, cantaloupe, raw. *Availble at https://ndb.nal.usda.gov/ndb/foods/show/09181?fgcd=&manu=&format=&count=&max=25&offset=&sort=default&order=asc&qlookup=cantaloupe&ds=&qt=&qp=&qa=&qn=&q=&ing= Accessed on May 27th, 2019.*

USDA. (2018c). USDA national nutrient database for standard reference: basic report: 11143, celery, raw. *Availble at: https://ndb.nal.usda.gov/ndb/foods/show/11143?fgcd=&manu=&format=&count=&max=25&offset=&sort=default&order=asc&qlookup=celery&ds=&qt=&qp=&qa=&qn=&q=&ing= Accessed on May 27th, 2019.*

USDA. (2018d). USDA national nutrient database for standard reference: basic report: 11205, cucumber,with peel, raw. *Availble at: https://ndb.nal.usda.gov/ndb/foods/show/11205?fgcd=&manu=&format=&count=&max=25&offset=&sort=default&order=asc&qlookup=cucumber&ds=&qt=&qp=&qa=&qn=&q=&ing= Accessed on May 27th, 2019.*

USDA. (2018e). USDA national nutrient database for standard reference: basic report: 11529, tomatoes, red, ripe, raw, year yound average. *Availble at: https://ndb.nal.usda.gov/ndb/foods/show/11529?fgcd=&manu=&format=&count=&max=25&offset=&sort=default&order=asc&qlookup=Tomatoes%2C+red%2C+ripe%2C+raw%2C+year+round+average&ds=&qt=&qp=&qa=&qn=&q=&ing= Accessed on May 27th, 2019.*

Vandamm, J. P., Li, D., Harris, L. J., Schaffner, D. W., & Danyluk, M. D. (2013). Fate of *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* on fresh-cut celery. *Food Microbiology*, *34* (1), 151-157.

Whiteley, A. T., Ruhland, B. R., Edrozo, M. B., & Reniere, M. L. (2017). A redox-responsive transcription factor is critical for pathogenesis and aerobic growth of *Listeria monocytogenes*. *Infection and Immunity, 85*(5), e00978-00916.

Yao, Y., & Ren, G. (2011). Effect of thermal treatment on phenolic composition and antioxidant activities of two celery cultivars. *LWT-Food Science and Technology, 44*(1), 181-185.

Zhu, K., Ding, X., Julotok, M., & Wilkinson, B. J. (2005). Exogenous isoleucine and fatty acid shortening ensure the high content of anteiso-C15: 0 fatty acid required for low-temperature growth of *Listeria monocytogenes*. *Applied and Environmental Microbiology, 71*(12), 8002-8007.

Zhu, Q., Gooneratne, R., & Hussain, M. (2017). *Listeria monocytogenes* in fresh produce: Outbreaks, prevalence and contamination levels. *Foods, 6*(3), 21.

Zuber, P. (2004). Spx-RNA polymerase interaction and global transcriptional control during oxidative stress. *Journal of Bacteriology, 186*(7), 1911-1918.

CHAPTER 5

SUMMARY

In this dissertation, we studied mechanisms of *L. monocytogenes* transfer and adaptation on fresh-cut produce. Firstly, we interviewed three retail supermarkets and identified that the common use of gloves represents a potential route for cross contamination with foodborne pathogens. Therefore, we investigated factors that may influence cross contamination of fresh-cut produce with *L. monocytogenes* and developed mathematical models to illustrate *L. monocytogenes* transfer. We found that contact times and contact pressures showed no significant difference on *L. monocytogenes* transfer between fresh-cut cantaloupe flesh and from cantaloupe surface (stem scar tissue and rind) to flesh. However, glove materials appeared to affect *L. monocytogenes* transfer from stem scar to cantaloupe flesh ($P = 0.0371$). No significant difference was observed in the transfer of *L. monocytogenes* from rind or flesh to flesh via different gloves materials. Residual surface liquids from pre-cut washing of whole cantaloupes resulted in a significantly higher transfer from cantaloupe rind to the flesh ($P = 0.0006$). Then, we developed and compared the quantitative models that can describe *L. monocytogenes* transfer on cantaloupe pieces via gloves. The predictive model showed that transfer of *L. monocytogenes* via gloves to fresh-cut cantaloupes could persist over 85 pieces. The results we obtained could provide insightful information to help retailers conduct meaningful risk assessments of practices used to prepare fresh-cut cantaloupes.

Secondly, we investigated the survival and growth kinetics of *L. monocytogenes* on eight types of fresh-cut produce juice. Results showed that the change of *L. monocytogenes*

populations in produce juices displayed a direct correlation with the juice pH values (R^2=0.83 at 10^5 CFU/ml and R^2=0.71 at 10^9 CFU/ml). At the refrigeration temperature, the population of *L. monocytogenes* decreased in apple juice, remained stable in tomato and cucumber juices, and increased in celery, cantaloupe, spinach, honeydew, and corn juices. With the information given, some recommendations for the storage time of the fresh-cut produce juice can be provided.

Also, we found that the fates of *L. monocytogenes* on fresh-cut produce and fresh-cut produce juice are different, which suggested that fresh produce juice may not be an appropriate surrogate to study the transcriptome of *L. monocytogenes* on fresh-cut produce. Using RNA-seq, we studied the transcriptomic response of *L. monocytogenes* on fresh-cut apple, cucumbers, cantaloupes, tomatoes, and celery to probe produce factors and bacterial mechanisms underlying *L. monocytogenes* adaptation on fresh-cut produce. We identified a core transcriptome that included commonly up- and down-regulated genes in *L. monocytogenes* on fresh-cut produce. We found that up-regulated gene sets involved amino acid metabolism and fatty acid metabolism while down-regulated gene sets involved carbon metabolism. Produce properties, such as amino acids, carbohydrates, pH, and antimicrobial substances, appeared to be correlated with *L. monocytogenes* transcriptome profile on fresh-cut produce. These results provided a detailed molecular assessment of metabolic and stress responses that may be important for *L. monocytogenes* adaptation on fresh-cut produce.

APPENDIX

SUPPLEMENTAL TABLES

All the supplemental tables (Table S1-Table S10) were uploaded to the public available website. The link of the website is:

https://drive.google.com/open?id=1rv7bRDo6IXWdv0_pgOzbI4h_Gn0X8CGD

Table S1. List of genes that showed significant up-regulation ($log_2FC \geq 1$, FDR<0.05) in transcript levels on all fresh-cut produce compared to genes in *L. monocytogenes* in PBS

Table S2. List of genes that showed significant up-regulation ($log_2FC \leq -1$, FDR<0.05) in transcript levels on all fresh-cut produce compared to genes in *L. monocytogenes* in PBS

Table S3. List of genes that showed significant up-regulation ($log_2FC \geq 1$, FDR<0.05) on specific fresh-cut produce compared to genes in *L. monocytogenes* in PBS

Table S4. List of genes that showed significant down-regulation ($log_2FC \leq -1$, FDR<0.05) on specific fresh-cut produce compared to genes in *L. monocytogenes* in PBS

Table S5. List of significantly up-regulated genes ($log_2FC \geq 1$, FDR<0.05) in *L. monocytogenes* on fresh-cut cantaloupes versus fresh-cut apples and fresh-cut celery

Table S6. List of significantly down-regulated genes ($log_2FC \leq -1$, FDR<0.05) in *L. monocytogenes* on fresh-cut cantaloupes versus fresh-cut apples and fresh-cut celery

Table S7. List of significantly up-regulated genes ($log_2FC \geq 1$, FDR<0.05) in *L. monocytogenes* on fresh-cut celery versus fresh-cut apples and fresh-cut cantaloupes

Table S8. List of significantly down-regulated genes ($log_2FC \leq -1$, FDR<0.05) in *L. monocytogenes* on fresh-cut celery versus fresh-cut apples and fresh-cut cantaloupes

Table S9. List of significantly up-regulated genes ($log_2FC \geq 1$, FDR<0.05) in *L. monocytogenes* on fresh-cut apples versus fresh-cut cantaloupes and fresh-cut celery

Table S10. List of significantly down-regulated genes ($log_2FC \leq -1$, FDR<0.05) in *L. monocytogenes* on fresh-cut apples versus fresh-cut cantaloupes and fresh-cut celery